



UTILITY

PATENT APPLICATION
TRANSMITTAL

Only for nonprovisional applications under 37 CFR § 1.53(b)

Attorney Docket No.

190106.407C1

SB Listing

First Inventor or Application Identifier

Andrzej Kilian and David Bowtell

Title

VERTEBRATE TELOMERASE GENES AND PROTEINS AND
USES THEREOF

Express Mail Label No.

EL48780827SUS

APPLICATION ELEMENTS

ADDRESS TO:

Box Patent Application
Assistant Commissioner for Patent
Washington, D.C. 20231

See MPEP chapter 600 concerning utility patent application contents.

- 1.
- ☒
- General Authorization Form & Fee Transmittal
-
- (Submit an original and a duplicate for fee processing)

- 2.
- ☒
- Specification [Total Pages]
- 71
-
- (preferred arrangement set forth below)

- Descriptive Title of the Invention
- Cross References to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to Microfiche Appendix
- Background of the Invention

- Brief Summary of the Invention
- Brief Description of the Drawings (if filed)
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

- 3.
- ☒
- Drawing(s) (35 USC 113) [Total Sheets]
- 53

4. Oath or Declaration [Total Pages]
- X

- a. ☐ Newly executed (original or copy)
- b. ☒ Copy from a prior application (37 CFR 1.63(d))
(for continuation/divisional with Box 17 completed)
- i. ☐ **DELETION OF INVENTOR(S)**
Signed statement attached deleting
inventor(s) named in the prior application,
see 37 CFR 1.63(d)(2) and 1.33(b)

- 5.
- ☒
- Incorporation by Reference (useable if box 4b is checked) The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered to be part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

- 6.
- ☐
- Microfiche Computer Program (Appendix)
-
7. Nucleotide and Amino Acid Sequence Submission
-
- (if applicable, all necessary)

- a. ☐ Computer-Readable Copy
- b. ☒ Paper Copy of Sequence Listing from Parent Application (identical to computer copy)
- c. ☒ Declaration Regarding Paper Copy of Sequence Listing from Parent Application and Computer Readable Copy From Parent Application

ACCOMPANYING APPLICATION PARTS

8. ☐ Assignment Papers (cover sheet & document(s))
9. ☐ 37 CFR 3.73(b) Statement (when there is an assignee) ☐ Power of Attorney
10. ☐ English Translation Document (if applicable)
11. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
12. ☒ Preliminary Amendment
13. ☒ Return Receipt Postcard
14. ☐ Small Entity Statement(s) ☒ Statement filed in prior application, Status still proper and desired
15. ☐ Certified Copy of Priority Document(s)
(if foreign priority is claimed)
16. ☒ Other: Certificate of Express Mail; Check: Copy of Assignment from Andrzej Kilian to Cambia; Copy of Assignment from Cambia to Cambia Biosystems, LLC; Copy of Election and Power of Attorney by Cambia LLC; Copy of Assignment from David Bowtell to Peter MacCallum Cancer Institute; Copy of Election and Power of Attorney by Peter MacCallum Cancer Institute; Request to Use Computer Readable Form From Parent Application; Appointment of Associate Power of Attorney

17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information below and in a preliminary amendment

- ☒ Continuation ☐ Divisional ☐ Continuation-In-Part (CIP) of prior Application No.: 09/108,401, filed 6/30/98

Prior application information: Examiner Einar Stole, Ph.D.Group / Art Unit 1653

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Respectfully submitted,

TYPED OR PRINTED NAME William T. Christiansen, Ph.D.SIGNATURE William T. ChristiansenREGISTRATION NO. 44,614Date Feb. 11, 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Andrzej Kilian, Canberra, Australia; and
 David Bowtell, Coburg, Australia
 Filed : February 11, 2000
 For : VERTEBRATE TELOMERASE GENES AND PROTEINS AND
 USES THEREOF

Docket No. : 190106.407C1
 Date : February 11, 2000

Box Patent Application
 Assistant Commissioner for Patents
 Washington, DC 20231

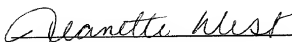
CERTIFICATE OF MAILING BY "EXPRESS MAIL"

Assistant Commissioner for Patents:

I hereby certify that the enclosures listed below are being deposited with the United States Postal Service "EXPRESS MAIL Post Office to Addressee" service under 37 C.F.R. § 1.10, Mailing Label Certificate No. EL487808275US, on February 11, 2000, addressed to Box Patent Application, Assistant Commissioner for Patents, Washington, DC 20231.

Respectfully submitted,

Seed Intellectual Property Law Group PLLC


 Jeanette West/Susan Johnson

DDM:nrap

Enclosures:

Postcard
 Check
 Form PTO/SB/05
 General Authorization Under 37 C.F.R. § 1.136(a)(3) and Fee Transmittal (+ copy)
 Specification, Claims, Abstract (71 pages)
 53 Sheets of Drawings (Figures 1A-15D)
 Copy of Declaration
 Copy of Assignment from Andrzej Kilian to Cambia
 Copy of Assignment from Cambia to Cambia Biosystems LLC
 Copy of Assignment from David Bowtell to Peter MacCallum Cancer Institute
 Copy of Election and Power of Attorney by Peter MacCallum Cancer Institute
 Paper Copy of Sequence Listing from Parent Application
 Request to Use Computer Readable Form From Parent Application
 Declaration Regarding Sequence Listing
 Appointment of Associate Power of Attorney
 Preliminary Amendment

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Present Application:

Applicants : Andrzej Kilian and David Bowtell
 Title : VERTEBRATE TELOMERASE GENES AND PROTEINS AND
 USES THEREOF
 Docket No. : 190106.407C1
 Date : February 11, 2000

Prior Application:

Examiner : Einar Stole, Ph.D.
 Art Unit : 1653
 Application No. : 09/108,401

Box Patent Application
 Assistant Commissioner for Patents
 Washington, DC 20231

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents:

Please amend the above-identified application as follows:

-- CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of pending United States Patent Application No. 09/108,401, filed June 30, 1998; which application claims the benefit of United States Provisional Application Nos. 60/051,410, filed July 1, 1997; 60/053,018, filed July 19, 1997; 60/053,329, filed July 21, 1997; 60/054,642, filed August 4, 1997; and 60/058,287, filed September 9, 1997, all of which are incorporated by reference in their entirety.—

In the Specification:

Amend the specification by inserting a new section before the "Technical Field" as follows:

In the Specification:

Please insert the enclosed Sequence Listing immediately after the Table of Contents ending on page 73.

Page 5, line 28, please delete "(SEQ ID NO:___)" and insert therefor -- (SEQ. ID NO:1) --.

Page 5, line 29, please delete "(SEQ ID NO:___)" and insert therefor -- (SEQ. ID NO:2) --.

Page 6, lines 1 and 2, please delete "(SEQ ID NO:___)" and insert therefor -- (SEQ. ID NO:3) --.

Page 6, line 2, please delete "(SEQ ID NO:___)" and insert therefor -- (SEQ. ID NO:4) --.

Page 6, line 3, immediately after "29-1132" please insert -- ;SEQ. ID NO:5 --.

Page 7, lines 9 and 10, immediately after "RNA variants" please insert -- (SEQ. ID NOs: 6-17) --.

Page 7, line 11, please delete "(SEQ ID NO:___)" and insert therefor -- (SEQ. ID NOs: 16 and 17) --.

Page 7, line 25, immediately after "telomerase" please insert -- (SEQ. ID NOs: 18-33) --.

Page 7, line 27, immediately after "proteins" please insert -- (SEQ. ID NOs: 34-86 and 155) --.

Page 7, line 30, immediately after "plasmid" please insert -- (SEQ. ID NO:87) --.

Page 8, line 2, immediately after "plasmid" please insert -- (SEQ. ID NO:88) --.

Page 8, line 4, immediately after "plasmid" please insert -- (SEQ. ID NO:89) --.

Page 8, line 12, please delete "(SEQ ID No. ___)" and insert therefor -- (SEQ. ID NO:2) --.

Page 20, line 27, immediately after "(AVRIRGKS" please insert -- ; SEQ. ID NO:90) --.

Page 20, line 28, immediately after "AXXXXGK(S)" please insert -- (SEQ. ID NO:91) --.

Page 21, line 17, immediately after "SQPEMEPPRRPSGCVG" please insert -- (SEQ. ID NO:92) --.

Page 21, line 18, immediately after "(PXXXXPXX)" please insert -- ; SEQ. ID NO:93) --.

Page 21, line 20, immediately after "HAGPPSTSRPPRPWDTP" please insert -- (SEQ. ID NO:94) --.

Page 36, line 6, immediately after "N₃XN*GUC(N_{3,6})" please insert -- (SEQ. ID NO:95) --.

Page 49, line 9, immediately after "AGGAGATCTCGCGATGCCGCGCGC TC-3'" please insert -- (SEQ. ID NO:96) --.

Page 49, line 10, immediately after "TCCACGCGTCTGCCCGGGTG-3'" please insert -- (SEQ. ID NO:97) --.

Page 49, line 27, immediately after "GCTGGTGCAGCGCGGGGACC" please insert -- (SEQ. ID NO:98) --.

Page 49, line 28, immediately after "CACAAGCTTGAATTCACATCTC ACCATGAAGGAGCTGGTGGCCGAGT" please insert -- (SEQ. ID NO:99) --.

Page 49, line 29, immediately after "GGCACGCACACCAGGCACTG" please insert -- (SEQ. ID NO:100) --.

Page 49, line 30, immediately after "CCTGCCTGAAGGAGCTGGTG" please insert -- (SEQ. ID NO:101) --.

Page 49, line 31, immediately after "GGACACCTGGCGGAAGGAG" please insert -- (SEQ. ID NO:102) --.

Page 49, line 32, immediately after "CCGAGTGCTGCAGAGGCTGT" please insert -- (SEQ. ID NO:103) --.

Page 49, line 33, immediately after "GAAGCCGAAGGCCAGCACGTT CT" please insert -- (SEQ. ID NO:104) --.

Page 49, line 34, immediately after "GTGCAGTGCTCCGCCAGCAC A" please insert -- (SEQ. ID NO:105) --.

Page 49, line 35, immediately after "GTTCCCAAGCAGCTCCAGAAAC AG" please insert -- (SEQ. ID NO:106) --.

Page 49, line 36, immediately after "GGCAGTGCCTTGTAGGAGCA" please insert -- (SEQ. ID NO:107) --.

Page 49, line 37, immediately after "CACTGGCTGATGAGTGTGTAC" please insert -- (SEQ. ID NO:108) --.

Page 50, line 1, immediately after "GACGTACACACTCATCAGCCA G"
please insert -- (SEQ. ID NO:109) --.

Page 50, line 2, immediately after "GGTCTTTCTTTTATGTCACGGA G"
please insert -- (SEQ. ID NO:110) --.

Page 50, line 3, immediately after "CACTTGAAGAGGGTGCAGCT" please
insert -- (SEQ. ID NO:111) --.

Page 50, line 4, immediately after "GTCTCACCTCGAGGGTGAAG" please
insert -- (SEQ. ID NO:112) --.

Page 50, line 5, immediately after "TTCACCCTCGAGGTGAGACGC T" please
insert -- (SEQ. ID NO:113) --.

Page 50, line 6, immediately after "TCGTAGTTGAGCACGCTGAAC" please
insert -- (SEQ. ID NO:114) --.

Page 50, line 7, immediately after "GCCTGAGCTGTACTTTGTCAA" please
insert -- (SEQ. ID NO:115) --.

Page 50, line 8, immediately after "CTGAGCTGTACTTTGTCAAGGA CA"
please insert -- (SEQ. ID NO:116) --.

Page 50, line 9, immediately after "GTACATGCGACAGTTCGTGGCT CA"
please insert -- (SEQ. ID NO:117) --.

Page 50, line 10, immediately after "CATGAAGCGTAGGAAGACGTC
GAAGA" please insert -- (SEQ. ID NO:118) --.

Page 50, line 11, immediately after "CGCAAACAGCTTGTCTCCATG TC"
please insert -- (SEQ. ID NO:119) --.

Page 50, line 12, immediately after "CTATGCCCGGACCTCCATCAGA"
please insert -- (SEQ. ID NO:120) --.

Page 50, line 13, immediately after "CTGATGGAGGTCCGGGCATAG" please
insert -- (SEQ. ID NO:121) --.

Page 50, line 14, immediately after "CCTCCGAGGCCGTGCAGT" please insert
-- (SEQ. ID NO:122) --.

Page 50, line 15, immediately after "CACCTCAAGCTTTCTAGATCAGT
CCAGGATGGTCTTGAAGTCA" please insert -- (SEQ. ID NO:123) --.

Page 50, line 16, immediately after "GGAAGGCAAAGGAGGGCAGGGC GA"
please insert -- (SEQ. ID NO:124) --.

Page 50, line 17, immediately after "CACGAATTCGGATCCAAGCTTT
TTTTTTTTTTTTTTT" please insert -- (SEQ. ID NO:125) --.

Page 50, line 18, immediately after "GGGTTGCGGAGGGTGGGC" please insert -- (SEQ. ID NO:126) --.

Page 50, line 19, immediately after "GCAGTGGTGAGCCGAGTCCTG" please insert -- (SEQ. ID NO:127) --.

Page 50, line 20, immediately after "CGACTTTGGAGGTGCCTTCA" please insert -- (SEQ. ID NO:128) --.

Page 50, line 22, immediately after "GCTGGTGACGCGGGGACC" please insert -- (SEQ. ID NO:129) --.

Page 50, line 23, immediately after "GAGGTGCAGAGCGACTACTCC A" please insert -- (SEQ. ID NO:130) --.

Page 50, line 24, immediately after "GTCTCACCTCGAGGGTGAAG" please insert -- (SEQ. ID NO:131) --.

Page 50, line 25, immediately after "GGCTGCTCCTGCGTTTGGTGG A" please insert -- (SEQ. ID NO:132) --.

Page 50, line 26, immediately after "GCCAGAGATGGAGCCACCC" please insert -- (SEQ. ID NO:133) --.

Page 50, line 27, immediately after "GGGTGGCTCCATCTCTGGC" please insert -- (SEQ. ID NO:134) --.

Page 50, line 28, immediately after "CCGCACGCTCATCTTCCACGT" please insert -- (SEQ. ID NO:135) --.

Page 50, line 29, immediately after "GCTTGGGGATGAAGCGGTC" please insert -- (SEQ. ID NO:136) --.

Page 50, line 30, immediately after "CGCCTGAGCTGTACTTTGTCA" please insert -- (SEQ. ID NO:137) --.

Page 50, line 31, immediately after "CACCTCAAGCTTTCTAGATCA GCTAGCGGCCAGCCCAACTCCCT" please insert -- (SEQ. ID NO:138) --.

Page 50, line 32, immediately after "GCAGCACACATGCGTGAAACCT GT" please insert -- (SEQ. ID NO:139) --.

Page 50, line 33, immediately after "GTGTCAGAGATGACGCGCAGG AA" please insert -- (SEQ. ID NO:140) --.

Page 50, line 34, immediately after "ACCCACACTTGCCTGTCCTGAG T" please insert -- (SEQ. ID NO:141) --.

Page 50, line 36, immediately after "TGGGC" please insert -- (SEQ. ID NO:142) --.

Page 50, line 37, immediately after "CTGTAATACGACTCACTATAGG GTTGCGGAGGGTGGGC" please insert -- (SEQ. ID NO:143) --.

Page 50, line 39, immediately after "GAGCCGAGTCCTG" please insert -- (SEQ. ID NO:144) --.

Page 50, line 41, immediately after "GGATCCGCCGCAGAGCACCGTC TG" please insert -- (SEQ. ID NO:145) --.

Page 50, line 42, immediately after "CGAAGCTTTCAGTGGGCCGGCA TCTGAAC" please insert -- (SEQ. ID NO:146) --.

Page 50, line 43, immediately after "CGAAGCTTTCACAGGCCCAGCC CAACTCC" please insert -- (SEQ. ID NO:147) --.

Page 50, line 44, immediately after "GCGGATCCAGGCCACGTCCTA CGTC" please insert -- (SEQ. ID NO:148) --.

Page 50, line 45, immediately after "GCGGATCCGTTTCAGATGCCGGC CCAC" please insert -- (SEQ. ID NO:149) --.

Page 56, line 24, immediately after "(PXXXXPXXP;PEMEPPRRP)" please insert -- (SEQ. ID NOs: 93 and 150 respectively) --.

Page 51, line 15, immediately after "PEMEPPRRP" please insert -- (- SEQ. ID NOs: 93 and 150 respectively) --.

In the Claims:

Please add the following claims:

65. (New) An isolated nucleic acid molecule encoding a splice variant of a reference human telomerase, wherein the reference human telomerase has regions α (encoded by 36 bases located at nucleotides 2131-2166 of Figure 1) and β (encoded by 182 bases located at nucleotides 2286-2468 of Figure 1).

66. (New) The nucleic acid molecule of claim 65, wherein the splice variant of human telomerase lacks nucleotide sequence encoding RTase motifs A, B, C, and D.

67. (New) The nucleic acid molecule of claim 65, wherein the splice variant of human telomerase lacks nucleotide sequence encoding RTase motif A.

68. (New) The nucleic acid molecule of any one of claims 65-67, wherein the splice variant of human telomerase lacks nucleotide sequence encoding a P-loop motif.

69. (New) The nucleic acid molecule of any one of claims 65-68, wherein the splice variant of human telomerase lacks the C-terminal domain of the reference human telomerase.

70. (New) The nucleic acid molecule of any one of claims 65-69, wherein the splice variant of human telomerase has an altered C-terminus comprising sequence encoding a consensus SH3 binding site.

71. (New) The nucleic acid molecule of claim 65, wherein the nucleic acid molecule comprises one of the sequences presented in Figure 11 (SEQ ID Nos: 34, 36, 38, 41, 43, 45, 47, 49, 51, 55, 63, 67, 71, 75, 79, 83), a complement thereof, or a sequence that

hybridizes under normal stringency conditions to the sequence or its complement.

72. (New) The nucleic acid molecule of claim 65, wherein the nucleic acid molecule encodes one of the amino acid sequences presented in Figure 11 (SEQ ID Nos: 35, 37, 39, 42, 44, 46, 48, 50, 52-54, 56-58, 60-62, 64-66, 68-70, 72-74, 76-78, 80-82, 84-86), or variant thereof.

73. (New) The complement of the nucleic acid molecule of claim 65.

74. (New) The nucleic acid molecule of claim 65, wherein said molecule is a DNA molecule.

75. (New) The nucleic acid molecule of claim 65, wherein said molecule is an RNA or cDNA molecule.

76. (New) An expression vector, comprising a promoter operably linked to the nucleic acid molecule according to claim 65.

77. (New) The expression vector of claim 76, wherein the vector is selected from the group consisting of bacterial vectors, retroviral vectors, adenoviral vectors and yeast vectors.

78. (New) A host cell containing a vector according to claim 76.

79. (New) The host cell of claim 78, wherein the cell is selected from the group consisting of human cell, monkey cell, mouse cell, rat cell, yeast cell and bacterial cell.

80. (New) An isolated nucleic acid molecule comprising of any of the sequences presented in Figure 10 (SEQ ID Nos: 18, 23, 25, 27, 29, 30, 32, 33), or a complement

of one of the sequences, or a variant of the sequences or complements thereof.

81. (New) An isolated nucleic acid molecule encoding any of the amino acid sequences in SEQ ID Nos. 24, 26, 28, and 31 or variant thereof

82. (New) An expression vector, comprising a promoter operably linked to the nucleic acid molecule according to claim 81.

83. (New) The expression vector of claim 82, wherein the vector is selected from the group consisting of bacterial vectors, retroviral vectors, adenoviral vectors and yeast vectors.

84. (New) A host cell containing a vector according to claim 83.

85. (New) The host cell of claim 84, wherein the cell is selected from the group consisting of human cell, monkey cell, mouse cell, rat cell, yeast cell and bacterial cell.

86. (New) An oligonucleotide comprising 15-100 contiguous nucleotides of one of the sequences presented in Figure 10 (SEQ ID Nos: 18, 23, 25, 27, 29, 30, 32, 33) or the complements thereof.

87. (New) The oligonucleotide of claim 86, wherein the oligonucleotide is from 15 to 36 nucleotides long.

88. (New) The oligonucleotide of claim 86, wherein the oligonucleotide is from 20 to 50 nucleotides long.

89. (New) The oligonucleotide of claim 86, wherein the oligonucleotide is labeled.

90. (New) The oligonucleotide of claim 89, wherein the label is a radiolabel, a chemiluminescent label, or biotin.

91. (New) A pair of oligonucleotide primers that amplify sequence selected from the group consisting of region 1 (SEQ ID No: 23), region α (SEQ ID No: 25), region β (SEQ ID No: 27), region 2 (SEQ ID No: 29), region 3 (SEQ ID No: 30), region X (SEQ ID No: 32) or region Y (SEQ ID No: 18).

92. (New) A pair of oligonucleotide primers that amplify sequence of human telomerase containing a splice junction, wherein the primer pair flanks nucleotide 222, 1950, 2131-2166, 2287-2468, 2843, or 3157 as presented in Figure 1 (SEQ ID No: 1).

93. (New) A pair of oligonucleotide primers that amplify sequence of human telomerase containing a splice junction, wherein only one primer of each primer pair flanks nucleotide 222, 1950, 2131-2166, 2287-2468, 2843, or 3157 as presented in Figure 1 (SEQ ID No: 1) and the other primer of the pair has sequence corresponding to all or a portion of one of the sequences presented in Figure 10 (SEQ ID Nos: 18, 23, 25, 27, 29, 30, 32, 33) or complements thereof.

94. (New) A method of diagnosing cancer in a patient, comprising preparing tumor cDNA and amplifying the tumor cDNA using a pair of oligonucleotide primers that amplify sequence selected from the group consisting of region 1 (SEQ ID No: 23), region α (SEQ ID No: 25), region β (SEQ ID No: 27), region 2 (SEQ ID No: 29), region 3 (SEQ ID No: 30), region X (SEQ ID No: 32) or region Y (SEQ ID No: 18), wherein the pattern of amplification is indicative of a diagnosis of cancer.

95. (New) A method of diagnosing cancer in a patient, comprising preparing tumor cDNA and amplifying the tumor cDNA using a pair of oligonucleotide primers that amplify sequence of human telomerase containing a splice junction, wherein the primer pair

flanks nucleotide 222, 1950, 2131-2166, 2287-2468, 2843, or 3157 as presented in Figure 1 (SEQ ID No: 1), wherein the pattern of amplification is indicative of a diagnosis of cancer.

96. (New) A method of diagnosing cancer in a patient, comprising preparing tumor cDNA and amplifying the tumor cDNA using a pair of oligonucleotide primers that amplify sequence of human telomerase containing a splice junction, wherein only one primer of each primer pair flanks nucleotide 222, 1950, 2131-2166, 2287-2468, 2843, or 3157 as presented in Figure 1 (SEQ ID No: 1) and the other primer of the pair has sequence corresponding to all or a portion of one of the sequences presented in Figure 10 (SEQ ID Nos: 18, 23, 25, 27, 29, 30, 32, 33) or complements thereof.

97. (New) A method of determining a pattern of telomerase RNA expression in cells, comprising,
preparing cDNA from mRNA isolated from the cells,
amplifying the cDNA using primers that amplify a splice variant of nucleic acid encoding human telomerase and
detecting the amplified product by hybridization with all or part of the sequence of region 1 (SEQ ID No: 23), all or part of the sequence of region α (SEQ ID No: 25), all or part of the sequence of region β (SEQ ID No: 27), all or part of the sequence of region 2 (SEQ ID No: 29), all or part of the sequence of region 3 (SEQ ID No: 30), all or part of the sequence of region X (SEQ ID No: 32) or all or part of the sequence of region Y (SEQ ID No: 18);
therefrom determining the pattern of telomerase RNA expression.

98. (New) A method of diagnosing cancer in a patient by determining a pattern of telomerase RNA expression, comprising,
amplifying sequence of human telomerase from cDNA synthesized from tumor RNA using primers that amplify a splice variant of human telomerase, and
detecting the amplified product by hybridization with all or part of the sequence of region 1 (SEQ ID No: 23), all or part of the sequence of region α (SEQ ID No: 25), all or part

of the sequence of region β (SEQ ID No: 27), all or part of the sequence of region 2 (SEQ ID No: 29), all or part of the sequence of region 3 (SEQ ID No: 30), all or part of the sequence of region X (SEQ ID No: 32) or all or part of the sequence of region Y (SEQ ID No: 18),

therefrom determining the pattern of telomerase RNA expression, wherein the pattern is indicative of a diagnosis of cancer.

99. (New) The method of claim 98, further comprising comparing the pattern to a pattern obtained from a reference cancer.

100. (New) A nucleic acid molecule encoding a human telomerase that lacks RTase motifs A, B, C, and D.

101. (New) A nucleic acid molecule encoding a human telomerase that lacks RTase motif A.

102. (New) The nucleic acid molecule of either of claims 101 or 102, wherein the human telomerase lacks a P-loop motif.

103. (New) The nucleic acid molecule of either of claims 101 or 102, wherein the human telomerase has an altered C-terminal domain comprising a consensus SH3 binding site.

104. (New) The nucleic acid molecule of either one of claims 102 or 103, wherein the human telomerase lacks the C-terminal domain of the human telomerase presented in SEQ ID No. 2.

105. (New) A nucleic acid molecule encoding a human telomerase that lacks a P-loop motif.

106. (New) A nucleic acid molecule encoding a human telomerase that has an

altered C-terminal domain comprising a consensus SH3 binding site.

107. (New) A nucleic acid molecule encoding a human telomerase that lacks the C-terminal domain of the human telomerase presented in SEQ ID No. 2.

REMARKS

Claims 1-107 are pending in the instant application. Newly added claims 65-107 do not add new matter. Applicants respectfully request consideration of the new claims 65-107. Additionally, the enclosed Sequence Listing includes no new material, but merely adds sequence identifiers to the specification as required by 37 C.F.R. § 1.821(d), and bring this application into compliance with 37 C.F.R. §§ 1.821-1.825 and WIPO Standard 25. Allowance of this application is earnestly solicited.

Respectfully submitted,

Seed Intellectual Property Law Group PLLC



William T. Christiansen, Ph.D.
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U:\cambia\71

VERTEBRATE TELOMERASE GENES AND PROTEINS AND USES THEREOF

CROSS-RELATED APPLICATIONS

This application claims the benefit of United States Provisional
5 Application Nos. 60/051,410, filed July 1, 1997; 60/053,018, filed July 19, 1997;
60/053,329, filed July 21, 1997; 60/054,642, filed August 4, 1997; and 60/058,287,
filed September 9, 1997, all of which are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention relates generally to telomerases, and particularly to the
10 human telomerase gene and protein and uses for diagnostics and therapy.

BACKGROUND OF THE INVENTION

Non-circular chromosomes require a specialized mechanism for
maintaining chromosome ends after each cell division because the polymerases
responsible for replication of chromosomal DNA are unable to fully replicate linear
15 DNA molecules, creating an "end replicating problem." To meet this challenge,
eukaryotic cells depend upon an enzyme, telomerase, to add short, typically G-rich,
relatively conserved repeats onto chromosomal ends. These repeat structures are
termed telomeres.

The presence of telomeres is essential for cell viability. The absence of
20 even a single telomere leads to cell cycle arrest in yeast, a eukaryotic cell (Sandell and
Zakian, *Cell* 75:729, 1993). Telomeres shorten during replication; telomerase restores
the telomeres. Thus, as expected, telomerase activity is primarily detected in actively
dividing cells. As such, telomerase activity is constitutive in unicellular organisms and
is regulated in more complex organisms, relatively abundant in germline and embryonic
25 tissues and cells as well as tumor cells. In contrast, telomerase activity is difficult to
detect in normal somatic human tissues. Moreover, rather than cessation of replication
resulting in decreased telomerase, recent data indicate that telomerase inhibition might

be one of the critical events in this transition. The seemingly direct correlation of telomerase/replication activities have prompted much speculation that inhibitors of telomerase could be a "universal" cancer therapeutic, effective for essentially all tumor types, whereas stimulators of telomerase could overcome the observed natural
 5 senescence of normal cells.

Spurred by these models, characterization of telomerase for culmination in isolation and cloning of telomerase has been a high priority. The mechanism of telomere elongation has been shown to center on the G-rich strand of the telomeric repeats. This G-rich strand, which extends to the 3' end of the chromosome, is extended
 10 by telomerase, a ribonucleoprotein, from the RNA component, which acts as a template. Various components of this complex have been isolated and cloned. The RNA component of the complex has been isolated and cloned from many different organisms, including humans (Feng et al. *Science* 269: 1236, 1995), mice and other mammalian species, *Saccharomyces cerevisiae*, *Tetrahymena*, *Euplotes*, and *Oxytricha* (see, Singer
 15 and Gottschling. *Science*, 266: 404, 1994; Lingner et al. *Genes & Develop.* 8: 1984, 1994; and Romero and Blackburn. *Cell* 67: 343, 1994). Protein components have been relatively refractory to isolation. Recently, the nucleotide sequences of several protein components have been determined (an 80 kD/95 kD dimeric protein from *Tetrahymena*, WO 96/19580; and a 67 kD protein from humans, WO 97/08314).

20 The present invention discloses nucleotide and amino acid sequences of telomerase, uses of these sequences for diagnostics and therapeutic uses, and further provides other related advantages.

SUMMARY OF THE INVENTION

In one aspect, this invention generally provides isolated nucleic acid
 25 molecules encoding vertebrate telomerase (including variants thereof). Representative examples of vertebrates include mammals such as humans, old world monkeys (*e.g.*, macaques, chimps, and baboons), dogs, rats, and mice, as well as non-mammalian organisms such as birds. In a preferred embodiment, the nucleic acid molecule encoding a vertebrate telomerase is provided, wherein the nucleic acid molecule

comprises the sequence presented in Figure 1, or hybridizes under stringent conditions to the complement of the sequence presented in Figure 1, provided that the nucleic acid molecule is not EST AA281296.

In other preferred embodiments, the nucleic acid molecule comprises any
 5 of the sequences presented in Figure 11 or encodes any of the amino acid sequences presented in Figure 11, or hybridizes under normal stringency conditions to the complement of the sequences thereof, provided that the nucleic acid molecule is not EST AA281296. In other embodiments, the nucleic acid molecule comprises any of the sequences presented in Figure 10, or hybridizes under normal stringency conditions to
 10 the complement of the sequences thereof.

In another aspect, the invention provides an oligonucleotide comprising from 10 to 100 contiguous nucleotides from the sequence presented in Figure 1 or its complement and from 10 to 100 contiguous nucleotides from the sequences presented in Figure 10 or the complements thereof. The oligonucleotides may be labeled with a
 15 detectable label.

In yet another aspect, an expression vector is provided, comprising a heterologous promoter operably linked to a nucleic acid molecule of human telomerase. The vector may be selected from the group consisting of bacterial vectors, retroviral vectors, adenoviral vectors and yeast vectors. Host cells containing such vectors are
 20 also provided.

In another aspect, the invention provides an isolated protein comprising a human telomerase protein. The protein may comprise the amino acid sequence presented in Figure 1 or variant thereof or any of the amino acid sequences presented in Figure 11 or variant thereof. In a related aspect, the protein is a portion of a human
 25 telomerase protein, which may derive from the sequences presented in Figures 1 or 11. In preferred embodiments, the portion is from 10 to 100 amino acids long.

In other aspects, antibodies that specifically binds to human telomerase protein or portions are provided.

In a preferred aspect, an oligonucleotide (*e.g.*, a nucleic acid probe or
 30 primer) is provided that is capable of specifically hybridizing to a nucleic acid molecule

encoding a human telomerase under conditions of normal stringency. Within certain embodiments, the nucleic acid molecule has a detectable label. Within certain embodiments, the nucleic acid molecule is selected such that it does not hybridize to nucleotides 1624-2012 presented in Figure 1. Within certain embodiments of the invention, the nucleic acid probe or primer may differ from a wild-type telomerase sequence by one or more nucleotides.

In a related aspect, the invention provides a pair of oligonucleotide primers capable of specifically amplifying all or a portion of a nucleic acid molecule encoding human telomerase. In specific embodiments, the nucleic acid molecule comprises the sequence presented in Figure 1, Figure 11, or complements thereof. In preferred embodiments, the pair of primers is capable of specifically amplifying sequence comprising all or a part of region 1, region α , region β , region 2, region 3 region X or region Y. In a related aspect, the invention provides an oligonucleotide that hybridizes specifically to a nucleic acid sequence in region 1, region α , region β , region 2, region 3 region X or region Y.

Methods for diagnosing cancer in a patient are also provided. These methods comprise preparing tumor cDNA and amplifying the tumor cDNA using primers that specifically amplify human telomerase nucleic acid sequence, wherein the detection of telomerase nucleic acid sequences is indicative of a diagnosis of cancer. The amount of detected sequences may be compared to the amount of amplified telomerase sequence to a control, wherein increase telomerase nucleic acid sequences over the control is indicative of a diagnosis of cancer.

In yet another aspect, a method of determining a pattern of telomerase RNA expression in cells is provided, comprising preparing cDNA from mRNA isolated from the cells, amplifying the cDNA using primers according to claim 35, therefrom determining the pattern of telomerase RNA expression. In preferred embodiments, the method further comprises detecting the amplified product by hybridization with an oligonucleotide having all or part of the sequence of region 1, region α , region β , region 2, region 3 region X or region Y. These methods may be used to diagnose cancer in a patient, wherein the pattern is indicative of a diagnosis of cancer.

The invention also provides non-human transgenic animals whose cells contain a human telomerase gene that is operably linked to a promoter effective for the expression of the gene. In preferred embodiments, the animal is a mouse and the promoter is tissue-specific. In a related aspect, the invention provides a mouse whose cells have an endogenous telomerase gene disrupted by homologous recombination with a nonfunctional telomerase gene, wherein the mouse is unable to express endogenous telomerase.

The invention also provides inhibitors of human telomerase activity, as well as assays for identifying inhibitors of telomerase activity wherein the inhibitor binds to telomerase and is not a nucleoside analogue. The inhibitor may be an antisense nucleic acid complementary to human telomerase mRNA, a ribozyme and the like. The inhibitors may be used to treat cancer.

Also provided are methods for identifying an effector of telomerase activity, comprising the general steps of (a) adding a candidate effector to a mixture of telomerase protein, RNA component and template, wherein the telomerase protein is encoded by an isolated nucleic acid molecule as described above; (b) detecting telomerase activity, and (c) comparing the amount of activity in step (b) to the amount of activity in a control mixture without candidate effector, therefrom identifying an effector. Within further embodiments the effector is an inhibitor. With yet other embodiments the the nucleic acid molecule encodes human telomerase.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, various references are set forth below which describe in more detail certain procedures or compositions (*e.g.*, plasmids, etc.), and are therefore incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-E present a DNA sequence (SEQ ID No:) and predicted amino acid sequence (SEQ ID No:) of human telomerase.

Figure 2 presents an alignment of *Euplotes aediculatus* p123 (SEQ ID No:___), yeast (EST2) (SEQ ID No:___) and human (HT1) telomerase protein (amino acids 29-1132) sequences. Reverse transcriptase motifs are indicated. The region of high homology among all three proteins is defined as the Telomerase region. The sequences are aligned with ClustalW.

Figure 3 is a scanned image of a Northern analysis showing that the telomerase catalytic subunit is expressed in LIM 1215 colon carcinoma cells but not in CCD primary fibroblasts. An mRNA of approximately 3.8 kb hybridizes to the hT1 probe. An additional cross-hybridizing mRNA of higher molecular weight is indicated by the top arrowhead. Cross-hybridization to ribosomal RNA present in the polyA⁺ RNA preparation is indicated. The same blot is also hybridized to a probe from the GAPDH gene as a loading control (lower panel). Marker sizes are indicated in kb.

Figure 4 is a scanned image of a Southern analysis showing that the telomerase catalytic subunit is encoded by a single gene and is not amplified in LIM 1215 cells. Genomic DNA isolated from peripheral human blood and LIM 1215 cell line is probed with a hT1 probe. The blot also contains dilutions of probe plasmid to control for the sensitivity of detection. The plasmid is diluted to approximately 10, 5 and 1 genome equivalents. H, *Hind* III; E, *Eco* RI; P, *Pst* I; X, *Xba* I; B, *Bam* HI.

Figure 5 shows the results of amplification of cDNAs synthesized from various tissues. Amplification is performed using primers from the hT1 cDNA sequence that span an intron in the hT1 gene, and the products are blotted and probed with a radiolabeled oligonucleotide from the hT1 sequence. Amplification is also performed on the same samples with a pair of primers from the β -actin gene as a loading control. a: hT1 cDNA control; b: human genomic DNA control; c: no template control; d: normal colon RNA; e: normal testis RNA; f: normal lymphocyte RNA; g: melanoma RNA (cerebral metastasis); h: melanoma RNA (subcutaneous ankle metastasis); i: melanoma RNA (liver metastasis); j: melanoma RNA (lung metastasis); k: melanoma RNA (axillary lymph node metastasis); l: melanoma RNA (skin metastasis); m: breast carcinoma RNA; n: breast carcinoma RNA; o: breast carcinoma RNA; p: breast carcinoma RNA.

Figure 6 presents results showing hT1 expression in pre-crisis cells and post-crisis cell lines. Upper panel: Nested amplification using primers within the original EST. Lower panel: Control RT-PCR using β -actin primers. a: BET-3K passage (p) 7 (pre-crisis); b: BET-3K p32 (post-crisis); c: BFT-3K p14 (pre-crisis); d: BFT-3K p 22 (post-crisis); e: BFT-3B p15 (pre-crisis); f: BFT-3B p29 (post-crisis); g: GM897 (ALT); h: IIICF/c (ALT); i: IIICF-T/B1 (ALT); j: No template control.

Figures 7A-C show some alternative splicing patterns of the hT1 transcript. A, Schematic representation of six splicing variants. B, Combinations of some identified RNA variants. C, Sequences of putative exon/intron junctions of RNA variants. Variants are marked as in part A. A complete DNA sequence (with protein translation) (SEQ ID No:) of variant 3 is presented. Amino acids corresponding to a potential c-Abl/SH3 binding site are underlined. Putative exon/intron junctions are marked with | and sequence coordinates are as in Figure 1. Putative spliced exons are in lower case and putative unspliced introns are in bold.

Figure 8 shows various splicing patterns of hT1 transcript in different tumor samples. Nested amplification (14 cycles) is performed using HT2026F and HT2482R primers on primary RT-PCR products generated with HT1875F and HT2781R primers. a: Lung carcinoma; b: Lymphoma; c: Lung carcinoma; d: Medulloblastoma; e: Lymphoma; f: Lymphoma; g: T47D; h: Pheochromocytoma; i: Lymphoma; j: Glioma; k: Lymphoma; l: No template control.

Figure 9 shows the results of amplification on cDNA synthesized from LIM 1215 cDNA. As shown, reverse transcriptase motif A is deleted from splicing variants containing sequence α . Primer combinations are: a, HTM2028F + HT2356R; b, HT2026F + HT2482R; c, HTM2028F + HT2482R; d, HT2026F + HT2482R.

Figures 10A-B present DNA sequences of variant regions of telomerase.

Figures 11A-W presents DNA and amino acid sequences of exemplary variant telomerase proteins.

Figure 12 is a scanned image of a telomerase activity assay.

Figures 13A-D present a schematic diagram of plasmid pAK128.4 and the DNA sequence of the plasmid.

Figures 14A-E present a schematic diagram of plasmid pAK128.7 and the DNA sequence of the plasmid.

Figures 15A-D present a schematic diagram of plasmid pAK128.14 and the DNA sequence of the plasmid.

5 DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention, it may be helpful to an understanding thereof to define certain terms used herein.

As used herein, "wild-type telomerase" generally refers to a polypeptide that enzymatically synthesizes nucleic acid sequences comprising simple repeat sequences (e.g., CCCTAA. see Zakian. *Science* 270: 1601, 1995) to ends of chromosomes. The amino acid sequence of one representative wild-type telomerase from human has been deduced and is presented in Figure 1 (SEQ ID No. ____). Within the context of this invention, it should be understood that telomerases of this invention include not only wild-type protein, but also variants (including alleles) of the wild-type protein sequence. Such variants may not necessarily exhibit enzymatic function. Briefly, such variants may result from natural polymorphisms, including RNA splice variants, generated by genetic recombination, or be synthesized by recombinant methodology, and moreover, may differ from wild-type protein by one or more amino acid substitutions, insertions, deletions, rearrangements or the like. Typically, when the result of synthesis, amino acid substitutions are conservative, i.e., substitution of amino acids within groups of polar, non-polar, aromatic, charged, etc. amino acids. In the region of homology to the wild-type sequence in the RTase motif regions variants will preferably have at least 90% amino acid sequence identity, and within certain embodiments, greater than 92%, 95%, or 97% identity. Outside the RTase motif region, variants will preferably have 75% amino acid identity, and within certain embodiments, at least 80%, 85%, 90%, 92%, 95% or 97% identity.

As will be appreciated by those skilled in the art, a nucleotide sequence encoding telomerase may differ from the wild-type sequence presented in the Figures; due to codon degeneracies, nucleotide polymorphisms, or amino acid differences. In

other embodiments, variants should preferably hybridize to the wild-type nucleotide sequence at conditions of normal stringency, which is approximately 25-30°C below T_m of the native duplex (e.g., 1 M Na⁺ at 65°C; 5X SSPE, 0.5% SDS, 5X Denhardt's solution, at 65°C or equivalent conditions; *see generally*, Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Press, 1987; Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing, 1987). T_m for other than short oligonucleotides can be calculated by the formula $T_m = 81.5 + 0.41\%(G+C) - \log(Na^+)$. Low stringency hybridizations are performed at conditions approximately 40°C below T_m , and high stringency hybridizations are performed at conditions approximately 10°C below T_m . Variants preferably have at least 75% nucleotide identity to wild-type sequence in the RTase motif region, preferably at least 80%, 85%, and most preferably at least 90% nucleotide identity.

As used herein, a "promoter" refers to a nucleotide sequence that contains elements that direct the transcription of a linked gene. At minimum, a promoter contains an RNA polymerase binding site. More typically, in eukaryotes, promoter sequences contain binding sites for other transcriptional factors that control the rate and timing of gene expression. Such sites include TATA box, CAAT box, POU box, AP1 binding site, and the like. Promoter regions may also contain enhancer elements. When a promoter is linked to a gene so as to enable transcription of the gene, it is "operatively linked".

An "isolated nucleic acid molecule" refers to a polynucleotide molecule in the form of a separate fragment or as a component of a larger nucleic acid construct, that has been separated from its source cell (including the chromosome it normally resides in) at least once in a substantially pure form. Nucleic acid molecules may be comprised of a wide variety of nucleotides, including DNA, RNA, nucleotide analogues, or some combination of these.

I. TELOMERASE, TELOMERASE GENES AND GENE PRODUCTS

As noted above, the invention provides compositions relating to vertebrate telomerase genes and gene products, and methods for the use of the genes

and gene products. Given the disclosure provided herein, a telomerase gene can be isolated from a variety of cell types that express telomerase, including immortalized or transformed cells. As exemplified herein, a cDNA and variants encoding telomerase from human cells are identified, isolated, and characterized. Telomerase protein is then readily produced by host cells transfected with an expression vector encoding telomerase.

A. Isolation of telomerase gene

As described herein, the invention provides genes encoding telomerase. Within one embodiment of the invention, a gene encoding human telomerase can be identified by amplification of a cDNA library using a primer pair designed from an EST sequence. The EST sequence GenBank Accession No. AA281296, is identified by sequence identity and similarity to a *Euplotes aediculatus* telomerase gene (GenBank accession no. U95964; Lingner et al., *Science* 276: 561, 1997). Sequence comparisons between the *Euplotes* telomerase gene and the EST show approximately 38% amino acid identity and 59% amino acid similarity.

Telomerase genes may be isolated from genomic DNA or cDNA. Genomic DNA is preferred when the promoter region or other flanking regions are desired. Genomic DNA libraries constructed in chromosomal vectors, such as YACs (yeast artificial chromosomes), bacteriophage vectors, such as λ EMBL3, λ gt10, cosmids, or plasmids, and cDNA libraries constructed in bacteriophage vectors (e.g., λ ZAPII), plasmids, or others, are suitable for screening. Such libraries may be constructed using methods and techniques known in the art (see Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, 1989) or purchased from commercial sources (e.g., Clontech, Palo Alto, CA). The DNA may be isolated from vertebrate cells, such as human cells, mouse cells, other rodent or primate cells, avian cells, and the like.

Within one embodiment, the telomerase gene is isolated by amplification using cDNA library DNA as templates. Using the reported EST sequence, human telomerase may be isolated. Briefly, sets of amplification primers are designed based upon the EST nucleotide sequence. Examples of such primers are presented in Table 2

(see also Example 1). Amplification of cDNA libraries made from cells with high telomerase activity is preferred. The primers described herein amplify a fragment that has a length predicted from the EST sequence from a LIM1215 cDNA library. LIM1215 is a human colon cancer cell line. Confirmation of the nature of the fragment is obtained by DNA sequence analysis.

DNA fragments encompassing additional sequence are amplified in reactions using a primer that hybridizes to vector sequence in conjunction with one of the EST primers. By using vector primers from either side of the cloning site in combination with the EST primers, a 1.6 kb fragment derived from the 3' region of h-TEL (human telomerase) and a 0.7 kb fragment derived from the 5' region are isolated. These fragments are verified as containing telomerase coding sequence by amplification with a pair of primers internal to the EST sequence. The two fragments are cloned into pBluescript and subjected to DNA sequence analysis. Additional DNA sequence is obtained by C-RACE and amplification procedures to obtain the 5' end of a cDNA as well as by hybridization and isolation of clones from the cDNA library.

The compiled DNA sequence and predicted amino acid sequence of a reference human telomerase are presented in Figure 1. As shown, the coding region of the reference telomerase is 3396 bases long and has an approximately 620 base long 3' untranslated region. The predicted amino acid sequence is 1132 amino acids long and may be delineated into four major domains: N-terminal, basic, reverse transcriptase (RT) and C-terminal. Furthermore, human telomerase contains regions of homology to other telomerases (*e.g.*, from *Euplotes* and *S. pombe*) and reverse transcriptases. These motifs are identified herein and in Kilian et al. (*Human Molecular Genetics*, 12: 2011-2019, 1997) as domains 1, 2, A, B, C, and D, in Nakamura et al., (*Science*, 277: 955-959) as domains 1, 2, A, B', C, D, and E, and in Meyerson et al. (*Cell*, 90: 785-795, 1997) as motifs 1-6. Regardless of the name used, these motifs encompass amino acids 621-626 (motif 1) and 631-634 (motif 2), 708-720 (motif A), 827-839 (motif B), 863-871 (motif C), and 895-902 (motif D). Because the boundaries of these motifs are based on similarity and identity with other telomerases, the functional boundary of each motif may be different.

In addition, variants of the reference telomerase sequence are obtained by amplifications, which are described herein. Their DNA and predicted amino acid sequences are presented in Figure 11 and discussed in further detail below. Briefly, some of these variants encode truncated proteins and others have different C-terminal sequences. These variants likely result from alternative RNA splicing because telomerase appears to be a single copy gene in humans (see Example 2).

Alternatively, other methods may be used to obtain a nucleic acid molecule that encodes telomerase. For example, a nucleic acid molecule encoding telomerase may be obtained from an expression library by screening with an antibody or antibodies reactive to telomerase (see, Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, NY, 1987; Ausubel et al. *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley-Interscience, NY, 1995). In another embodiment, nucleic acid molecules encoding telomerase may be isolated by hybridization screening of cDNA or genomic libraries. Oligonucleotides for hybridization screening may be designed based on the DNA sequence of human telomerase presented herein. Oligonucleotides for screening are typically at least 11 bases long and more usually at least 20 or 25 bases long. In one embodiment, the oligonucleotide is 20-30 bases long. Such an oligonucleotide may be synthesized in an automated fashion. To facilitate detection, the oligonucleotide may be conveniently labeled, generally at the 5' end, with a reporter molecule, such as a radionuclide. (e.g., ^{32}P), enzymatic label, protein label, fluorescent label, or biotin. A library is generally plated as colonies or phage, depending upon the vector, and the recombinant DNA is transferred to nylon or nitrocellulose membranes. Hybridization conditions are tailored to the length and GC content of the oligonucleotide. Following denaturation, neutralization, and fixation of the DNA to the membrane, membranes are hybridized with labeled probe. Suitable hybridization conditions may be found in Sambrook et al., *supra*. Ausubel et al., *supra*, and furthermore hybridization solutions may contain additives such as tetramethylammonium chloride or other chaotropic reagents or hybotropic reagents to increase specificity of hybridization (see for example, PCT/US97/17413). Following hybridization, suitable detection methods reveal

hybridizing colonies or phage that are then isolated and propagated. Candidate clones or amplified fragments may be verified as containing telomerase DNA by any of various means. For example, the candidate clones may be hybridized with a second, non-overlapping probe or subjected to DNA sequence analysis. In these ways, clones
 5 containing a telomerase gene or gene fragment, which are suitable for use in the present invention, are isolated.

Telomerase DNA may also be obtained by amplification of cDNA or genomic DNA. Oligonucleotide primers for amplification of a full-length cDNA are preferably derived from sequences at the 5' and 3' ends of the coding region.
 10 Amplification of genomic sequences will use primers that span intronic sequences and may use conditions that favor long amplification products (see Promega catalogue). Briefly, oligonucleotides used as amplification primers preferably do not have self-complementary sequences nor have complementary sequences at their 3' end (to prevent primer-dimer formation). Preferably, the primers have a GC content of about 50% and
 15 contain restriction sites to facilitate cloning. Generally, primers are between 15 and 50 nucleotides long, and more usually between 20 and 35 nucleotides long. The primers are annealed to cDNA or genomic DNA and sufficient amplification cycles are performed to yield a detectable product, preferably one that is readily visualized by gel electrophoresis and staining. The amplified fragment is purified and inserted into a
 20 vector (e.g., a viral, phagemid or plasmid vector, such as λ gt10 or pBS(M13+)) and propagated.

Telomerase genes from a multitude of species can be isolated using the compositions provided herein. For closely related species, the human sequence or portion thereof may be utilized as a probe on a genomic or cDNA library. For example,
 25 a fragment of the telomerase gene that encompasses the catalytic site (approximately corresponding to amino acids 605-915 of Figure 1) may be labeled and used as a probe on a library constructed from mouse, primate, rat, dog, or other vertebrate, warm-blooded, or mammalian species. An initial hybridization at normal stringency may yield clones or fragments encoding telomerase. If no hybridization is observed, relaxed
 30 (low) stringency hybridizations may be pursued. Guidelines for varying the stringency

of the hybridization may be acquired from Sambrook et al., *supra*, and other well-known sources. Such probes may also be used on libraries from evolutionarily diverse species, such as *Drosophila*, although hybridization conditions will typically be more relaxed.

5 Other methods may alternatively be used to isolate telomerase genes from non-human species. These methods include, but are not limited to, amplification using primers derived from conserved areas (e.g., RTase motifs), amplification using degenerate primers from various regions of telomerase including the RTase region, antibody probing of expression libraries, telomerase RNA probing of expression
10 libraries, and the like. A gene sequence is identified as a telomerase by amino acid similarity and / or nucleic acid identity. Generally, amino acid similarity, which allows for conservative differences, is preferred to identify a telomerase. From diverse species, amino acid similarity is generally at least 30% and preferably at least 40% or at least 50%. Nucleic acid identity may be lower and thus difficult to assess. Several readily
15 available computer analysis programs, such as BLASTN and BLASTP, are useful to determine relatedness of genes and gene products. Candidate telomerase genes are examined for enzyme activity by one of the functional assays described herein or other equivalent assays.

B. Variant telomerase genes

20 Variants (including alleles) of the telomerase nucleic acid or amino acid sequence provided herein may be readily isolated from natural variants (e.g., polymorphisms, splice variants, mutants), synthesized, or constructed. Depending upon the intended use, mutants may be constructed to exhibit altered or deficient telomerase function. Particularly useful telomerase genes encode a protein lacking enzyme activity
25 but that has a dominant negative phenotype. The telomerase variants, moreover, may lack one or more of known telomerase activities, including reverse transcriptase activity, nucleolytic activity, telomere binding activity, dNTP binding activity, and telomerase RNA (hTR) binding activity.

One skilled in the art recognizes that many methods have been
30 developed for generating mutants (see, generally, Sambrook et al., *supra*; Ausubel

et al., *supra*). Briefly, preferred methods for generating a few nucleotide substitutions utilize an oligonucleotide that spans the base or bases to be mutated and contains the mutated base or bases. The oligonucleotide is hybridized to complementary single stranded nucleic acid and second strand synthesis is primed from the oligonucleotide.

5 Similarly, deletions and/or insertions may be constructed by any of a variety of known methods. For example, the gene can be digested with restriction enzymes and religated such that some sequence is deleted or ligated with an isolated fragment having cohesive ends so that an insertion or large substitution is made. In another embodiment, variants are generated by "exon shuffling" (see U.S. Patent No. 5,605,793). Variant sequences

10 may also be generated by "molecular evolution" techniques (see U. S. Patent No. 5,723,323). Other means to generate variant sequences may be found, for example, in Sambrook et al. (*supra*) and Ausubel et al. (*supra*). Verification of variant sequences is typically accomplished by restriction enzyme mapping, sequence analysis, or probe hybridization, although other methods may be used. The double-stranded nucleic acid

15 is transformed into host cells, typically *E. coli*, but alternatively, other prokaryotes, yeast, or larger eukaryotes may be used. Standard screening protocols, such as nucleic acid hybridization, amplification, and DNA sequence analysis, will identify mutant sequences.

In preferred embodiments, variant telomerases are inactive with respect

20 to enzyme activity and impart a dominant negative phenotype to a host cell. Regardless of the actual mechanism, when a dominant negative telomerase is expressed in a cell, the native active telomerase is rendered inactive. In the catalytic domain, RTase motifs share conserved aspartic acid residues. Human telomerase also contains these critical residues: Asp 712, Asp 718, Asp 868, and Asp 869. Mutation of one or more of these

25 Asp residues to a non-conservative amino acid (e.g., alanine) will likely destroy enzymatic activity and or affect telomere shortening. For each of these mutants, dominant negativity is assayed. Preferred mutants are dominant negative and induce a senescence phenotype in certain embodiments. Other dominant negative variants may be generated by deletion of one or more of the RTase motifs or alteration of the region

30 involved in DNA priming (such as motif E), binding site for the RNA component, the

template binding site, the metal ion binding site (such as motif C), and the like.

In other embodiments, the nucleic acid molecule encoding telomerase may be fused to another nucleic acid molecule. As will be appreciated, the fusion partner gene may contribute, within certain embodiments, a coding region. Thus, it may be desirable to use only the catalytic site of telomerase (*e.g.*, amino acids 609-915), individual RTase motifs (described above), any of the splicing variant telomerases described herein, the telomerase RNA binding site and the like. The choice of the fusion partner depends in part upon the desired application. The fusion partner may be used to alter specificity of the telomerase, provide a reporter function, provide a tag sequence for identification or purification protocols, and the like. The reporter or tag can be any protein that allows convenient and sensitive measurement or facilitates isolation of the gene product and does not interfere with the function of the telomerase. For reporter function, β -glucuronidase (U.S. Patent No: 5,268,463), green fluorescent protein and β -galactosidase are readily available as DNA sequences. A peptide tag is a short sequence, usually derived from a native protein, which is recognized by an antibody or other molecule. Peptide tags include FLAG®, Glu-Glu tag (Chiron Corp., Emeryville, CA) KT3 tag (Chiron Corp.), T7 gene 10 tag (Invitrogen, La Jolla, CA), T7 major capsid protein tag (Novagen, Madison, WI), His₆ (hexa-His), and HSV tag (Novagen). Besides tags, other types of proteins or peptides, such as glutathione-S-transferase may be used.

C. Fragments and oligonucleotide derived from telomerase genes

In addition, portions or fragments of telomerase gene may be isolated or constructed for use in the present invention. For example, restriction fragments can be isolated by well-known techniques from template DNA, *e.g.*, plasmid DNA, and DNA fragments, including restriction fragments, can be generated by amplification. Furthermore, oligonucleotides can be synthesized or isolated from recombinant DNA molecules. One skilled in the art will appreciate that other methods are available to obtain DNA or RNA molecules having at least a portion of a telomerase sequence. Moreover, for particular applications, these nucleic acids may be labeled by techniques known in the art with a radiolabel (*e.g.*, ³²P, ³³P, ³⁵S, ¹²⁵I, ¹³¹I, ³H, ¹⁴C), fluorescent label

(e.g., FITC, Cy5, RITC, Texas Red), chemiluminescent label, enzyme, biotin and the like.

Methods for obtaining fragments are well-known in the art. Portions that are particularly useful within the context of this invention contain the catalytic site, individual RTase motifs, the putative intronic sequences (see Figure 10), and the like. Oligonucleotides are generally synthesized by automated fashion; methods and apparatus for synthesis are readily available (e.g., Applied Biosystems Inc, CA). Oligonucleotides may contain non-naturally occurring nucleotides, such as nucleotide analogues, a modified backbone (e.g., peptide backbone), nucleotide derivatives (e.g., biotinylated nucleotide), and the like. As used herein, oligonucleotides refers to a nucleic acid sequence of at least about 7 nucleotides and generally not longer than about 100 nucleotides. Usually, oligonucleotides are between about 10 and about 50 bases, more often between about 18 and about 35 nucleotides long. Oligonucleotides can be single-stranded or in some cases double-stranded. As used herein, portions of a nucleic acid refer to a polynucleotide that contains less than the entire parental nucleic acid sequence. For example, a portion of telomerase coding sequence contains less than a full-length telomerase sequence. A 'portion' is generally at least about seven nucleotides, and may be as many as 10, 20, 25 or more nucleotides in length. A fragment refers to a polynucleotide molecule of any length and can encompass an oligonucleotide, although more usually, but not to be limiting, the term oligonucleotide is used to denote short polynucleotides and the term fragment is used to denote longer polynucleotides.

Oligonucleotides for use as primers for amplification and probes for hybridization screening may be designed based on the DNA sequence of human telomerase presented herein. Oligonucleotide primers for amplification of a full-length cDNA are preferably derived from sequences at the 5' and 3' ends. Primers for amplification of specific regions are chosen to generate products of an easily detectable size. In preferred embodiments, primers are chosen that flank the sequences subject to alternative RNA splicing. In preferred embodiments, one set of primers is chosen such that both the product that spans spliced-in sequence as well as the product that spans

spliced-out sequence are suitable sizes to be detected under the same reaction conditions. In other embodiments, two sets of primers are used to detect the alternative spliced RNAs. For example, one set of primers flanks the splice junction in order to detect a spliced-out product. The second set of primers may be derived very close to the junction (such that a spliced-out amplification product is the same size or barely larger than a primer-dimer length) or one or more of the set may be derived from the spliced-in sequence (such that the spliced-out RNA would not yield any product). An amplified

Amplification primers preferably do not have self-complementary sequences nor have complementary sequences at their 3' end (to prevent primer-dimer formation). Preferably, the primers have a GC content of about 50% and may contain restriction sites to facilitate cloning. Amplification primers usually are at least 15 bases and usually are not longer than 50 bases, although in some circumstances and conditions shorter or longer lengths can be used. More usually, primers are from 17 to 40 bases long, 17 to 35 bases long, or 20 to 30 bases long. The primers are annealed to cDNA or genomic DNA and sufficient amplification cycles, generally 20-40 cycles, are performed to yield a product readily visualized by gel electrophoresis and staining or by hybridization. The amplified fragment can be purified and inserted into a vector, such as λ gt10 or pBS(M13-), and propagated, isolated and subjected to DNA sequence analysis, subjected to hybridization, or the like.

An oligonucleotide hybridization probe suitable for screening genomic, cDNA or other types (e.g., mutant telomerase sequences) of libraries, probing southern, northern, or northwestern blots, amplification products, and the like may be designed based on the sequences provided herein. Oligonucleotides for hybridization are typically at least 11 bases long, generally less than 100 bases long, and preferably at least 15 bases long, at least 20 bases long, at least 25 bases long, and preferably 20-70, 25-50, or 30-40 bases long. To facilitate detection, the oligonucleotide may be conveniently labeled, generally at the 5' end, with a reporter molecule, such as a radionuclide, (e.g., ^{32}P), enzymatic label, protein label, fluorescent label, or biotin. (see Ausubel et al., and Sambrook et al., *supra*). A library is generally plated as colonies or phage, depending upon the vector, and the recombinant DNA is transferred

to nylon or nitrocellulose membranes. Following denaturation, neutralization, and fixation of the DNA to the membrane, membranes are hybridized with labeled probe, and washed. Suitable detection methods reveal hybridizing colonies or phage that are then isolated and propagated. Methods for transferring nucleic acids to membranes and performing hybridizations are well known. In certain embodiments, additives to hybridization solution, such as a chaotrope (e.g., tetramethylammonium chloride) or a hybotrope (e.g., ammonium trichloroacetate; see PCT/ US97/17413) are added to increase sensitivity and specificity of hybridization. A probe specifically hybridizes to a nucleic acid if it remains detectably annealed after washing under conditions equivalent to hybridization conditions (expressed herein as the number of degrees less than T_m).

D. Splicing variants of human telomerase

In addition to the reference telomerase DNA and protein sequences presented in Figures 1, several RNA splice variants are observed. Although some of the variants may reflect incompletely processed mRNA, it is noteworthy that such variants are abundant in an RNA sample (LIM1215) preselected for polyadenylated mRNA. These findings, together with their clustering in the RT domain, suggest that the insertion variants more likely reflect regulation of hTl protein expression. For example, variants in which exons are deleted (see α , β , Fig. 7) are likely alternative mature coding for variant proteins. Additional evidence in support of alternative proteins comes from sequence analysis of cDNA clones identified in a LIM1215 cDNA library that contained both deletions and insertions compared to the reference sequence.

At least seven different putative introns appear to be retained in mRNAs (see Figure 7, which displays 6 of the 7 introns). The introns may be independently retained, thus, a particular mRNA may have none, any one, two, etc. up to seven introns. The maximum number of different mRNAs resulting from seven independently spliced introns is 2^7 , or 128 different mRNAs. DNA sequences of these introns are presented in Figure 10. The 5' most intron, called sequence "X", is an unknown length, and only a partial sequence is presented.

The reference telomerase sequence (Figure 1) includes intron α and intron β . In the following discussion, the effect of presence/absence and location of each intron is presented on the basis that it is the only alteration. It will be appreciated that a particular intron may alter the sequence of the translated product, regardless of whether other introns are spliced in or out. For example, the presence of intron 1 results in a frameshift and truncated protein, regardless of whether introns α , β , 2 or 3 are spliced in or out.

The presence of intron "X" results in a truncated protein that contains approximately 600 N-terminal amino acids and lacks all of the RTase motifs. The presence of intron "Y" at base 222 results in a frameshifted protein that terminates within three codons past the intron. As the Y intron is very GC rich, approximately 78%, which is difficult to sequence, it is possible that intron Y causes an insertion of about 35 amino acids and not a frameshift.

Intron 1 at nucleotide 1950 is 38 bp and its presence in mRNA causes a frame-shift and ultimate translation of a truncated protein (stop codon at nt 1973). This truncated protein contains only RTase domains 1 and 2.

Intron α , located from bases 2131-2166 is frequently observed spliced out of telomerase mRNA. A protein translated from such an RNA is deleted for 12 amino acids, removing RTase motif A. This motif appears to be critical for RT function; a single amino acid mutation within this domain in the yeast EST2 protein results in a protein that functions as a dominant negative and results in cellular senescence and telomere shortening.

Another of the variant sequences, the β -exon deletion at base 2286-2468, encodes a truncated protein, due to a reading frameshift at base 2287, which is joined to base 2469, and subsequently a termination codon at base 2605. This variant protein has RTase domains 1, 2, A, B, and part of C, but lacks another motif; in addition to the RTase domain motifs, another sequence motif (AVRIRGKS) identified in the β insert of hT1 matches a P-loop motif consensus AXXXXGK(S) (Saraste et al., *Trends Biochem. Sci.* 15, 430-434, 1990). This motif is found in a large number of protein families including a number of kinases, bacterial dnaA, recA, recF, mutS and ATP-

binding helicases (Devereaux et al., *Nucleic Acids Res.*, 12, 387-395, 1984). The P-loop is thus present only in a subpopulation of the h-TEL mRNAs in most RNA samples analyzed and completely absent from several tumor samples (Figure 8).

Intron 2 at base 2843 contains an in-frame termination codon, resulting in a truncated protein that has the entire RTase domain region, but lacks the C-terminus. As the C-terminus may play a regulatory role, protein activity will likely be affected. When intron 3 is retained, a smaller protein is also produced because the intron contains an in-frame stop codon. Thus, the protein has an altered C-terminal sequence. What activity such proteins might have is currently unknown. The crystal structure of the HIV-1 reverse transcriptase demonstrates that a short form of the protein (p51) that lacks the RNAase domain is inhibited by the C-terminal 'connection' folding into the catalytic cleft. If hT1 is assumed to adopt a similar structure to HIV-RT, then C-terminal hT1 protein variants may reflect a similar mechanism of regulation.

In addition to variants that lack the reference C-terminal domain, a variant with intron 3 at base 2157 expresses an alternative C-terminal domain. Furthermore, the coding region donated by intron 3 has a potential SH3 binding site, SGQPEMEPPRRP^{SG}CVG, which matches the consensus c-Abl SH3 binding peptide (PXXXXPXXP) found in proteins such as ataxia telangiectasia mutated (ATM). A second example of this motif is found within the N-terminal end of the hT1 protein in the peptide HAGPP^{TS}SRP^{PR}WDTP. Other alternative C-terminal domains are found in telomerase cDNAs: the EST12462 (GenBank Accession No. AA299878) has about 50 bases of identical sequence up to base 2157 and then diverges from the reference telomerase sequence as well as intron 3. This new sequence has an internal stop codon in 50 bases that would result in a truncated C-terminus.

The variant detected in one ALT cell line (Fig. 6, lane i) opens up the possibility that the basic domain of hT1 may contribute to the ALT mechanism in at least some ALT cell lines. Interestingly, this ALT cell line expresses the hTR gene. One possible mechanism of ALT could involve dysregulated telomerase components that are inactive in the TRAP assay.

The following table summarizes the splice variants and resulting proteins. For simplicity, only a single variant is listed for each resulting protein. Furthermore, as noted above, the presence of the Y intron appears to cause a frameshift resulting in a truncated protein, but may cause an insertion. Thus, each reading frame of the Y intron is presented and the table is constructed as if the insertion does not cause a truncated protein. An independent assortment of these known introns would lead to 128 different mRNA sequences. The DNA and amino acid sequences for the variants in Table 1 are presented in Figure 11.

Table 1

Protein	Insert sequences					
	Y	1	α	β	2	3
truncated #1	0	+	0	0	0	0
truncated #2	0	0	+	0	0	0
reference protein	0	0	+	+	0	0
truncated #3	0	0	+	+	+	0
altered C-terminus	0	0	+	+	0	+
lacks motif A	0	0	0	+	0	0
truncated #3; lacks motif A	0	0	0	+	+	0
lacks motif A: altered C-terminus	0	0	0	+	0	+
truncated #1 (ver 2)	-	+	0	0	0	0
truncated #2 (ver 2)	+	0	+	0	0	0
reference protein (ver 2)	-	0	+	+	0	0
truncated #3 (ver 2)	+	0	+	+	+	0
altered C-terminus (ver 2)	+	0	+	+	0	+
lacks motif A (ver 2)	+	0	0	+	0	0
truncated #3 (ver 2)	+	0	0	+	+	0
lacks motif A: altered C-terminus (ver 2)	-	0	0	+	0	+

E. Vectors, host cells and means of expressing and producing protein

Telomerase protein may be expressed in a variety of host organisms. In one embodiment, telomerase is produced in bacteria, such as *E. coli*, for which many expression vectors have been developed and are readily available. Other suitable host organisms include other bacterial species, and eukaryotes, such as yeast (e.g.,

Saccharomyces cerevisiae), mammalian cells (e.g., CHO and COS-7), and insect cells (e.g., Sf9).

A DNA sequence encoding telomerase, a portion thereof, a variant, fusion protein or the like, is introduced into an expression vector appropriate for the host. In certain embodiments, telomerase is inserted into a vector such that a fusion protein is produced. The telomerase sequence is derived from an existing fragment, cDNA clone, or synthesized. A preferred means of synthesis is amplification of the gene from cDNA using a set of primers that flank the coding region or the desired portion of the protein. As discussed above, the telomerase sequence may contain alternative codons for each amino acid with multiple codons. The alternative codons can be chosen as "optimal" for the host species. Restriction sites are typically incorporated into the primer sequences and are chosen with regard to the cloning site of the vector. If necessary, translational initiation and termination codons can be engineered into the primer sequences.

At minimum, the vector must contain a promoter sequence. Other regulatory sequences may be included. Such sequences include a transcription termination signal sequence, secretion signal sequence, origin of replication, selectable marker, and the like. The regulatory sequences are operationally associated with one another to allow transcription or translation.

The plasmids used herein for expression of telomerase include a promoter designed for expression of the proteins in a host cell (e.g., bacterial). Suitable promoters are widely available and are well known in the art. Inducible or constitutive promoters are preferred. Such promoters for expression in bacteria include promoters from the T7 phage and other phages, such as T3, T5, and SP6, and the *trp*, *lpp*, and *lac* operons. Hybrid promoters (see, U.S. Patent No. 4,551,433), such as *tac* and *trc*, may also be used. Promoters for expression in eukaryotic cells include the P10 or polyhedron gene promoter of baculovirus/insect cell expression systems (see, e.g., U.S. Patent Nos. 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784), MMTV LTR, CMV IE promoter, RSV LTR, SV40, metallothionein promoter (see, e.g., U.S. Patent No. 4,870,009) and other inducible promoters. For expression of the proteins, a

promoter is inserted in operative linkage with the coding region for the telomerase protein.

The promoter controlling transcription of the telomerase may itself be controlled by a repressor. In some systems, the promoter can be derepressed by altering the physiological conditions of the cell, for example, by the addition of a molecule that competitively binds the repressor, or by altering the temperature of the growth media. Preferred repressor proteins include, but are not limited to, the *E. coli* lacI repressor, which is responsive to IPTG induction, the temperature sensitive λ cI857 repressor, and the like. The *E. coli* lacI repressor is preferred.

In other preferred embodiments, the vector also includes a transcription terminator sequence, which has either a sequence that provides a signal that terminates transcription by the polymerase that recognizes the selected promoter and/or a signal sequence for polyadenylation.

Preferably, the vector is capable of replication in the host cells. Thus, when the host cell is a bacterium, the vector preferably contains a bacterial origin of replication. Preferred bacterial origins of replication include the fl-ori and col E1 origins of replication, especially the ori derived from pUC plasmids. In yeast, ARS or CEN sequences can be used to assure replication. A well-used system in mammalian cells is SV40 ori.

The plasmids also preferably include at least one selectable marker that is functional in the host. A selectable marker gene includes any gene that confers a phenotype on the host that allows transformed cells to be identified and selectively grown. Suitable selectable marker genes for bacterial hosts include the ampicillin resistance gene (Amp^r), tetracycline resistance gene (Tc^r) and the kanamycin resistance gene (Kan^r). The kanamycin resistance gene is presently preferred. Suitable markers for eukaryotes usually require a complementary deficiency in the host (e.g., thymidine kinase (tk) in tk- hosts). However, drug markers are also available (e.g., G418 resistance and hygromycin resistance).

The sequence of nucleotides encoding the telomerase may also include a secretion signal, whereby the resulting peptide is synthesized as precursor protein and

is subsequently processed and secreted. The resulting processed protein may be recovered from periplasmic space or fermentation medium. Secretion signals suitable for use are widely available and are well known in the art (von Heijne, *J. Mol. Biol.* 184:99-105, 1985). Prokaryotic and eukaryotic secretion signals that are functional in *E. coli* (or other host) may be employed. The presently preferred secretion signals include, but are not limited to, those encoded by the following *E. coli* genes: *peIB* (Lei et al., *J. Bacteriol.* 169:4379, 1987), *phoA*, *ompA*, *ompT*, *ompF*, *ompC*, beta-lactamase, and alkaline phosphatase.

One skilled in the art appreciates that there are a wide variety of suitable vectors for expression in bacterial cells and which are readily obtainable. Vectors such as the pET series (Novagen, Madison, WI), the *tac* and *trc* series (Pharmacia, Uppsala, Sweden), pTTQ18 (Amersham International plc, England), pACYC 177, pGEX series, and the like are suitable for expression of a telomerase. Baculovirus vectors, such as pBlueBac (*see, e.g.*, U.S. Patent Nos. 5,278,050, 5,244,805, 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784; available from Invitrogen, San Diego) may be used for expression of the telomerase in insect cells, such as *Spodoptera frugiperda* sf9 cells (*see*, U.S. Patent No. 4,745,051). The choice of a host for the expression of a telomerase is dictated in part by the vector. Commercially available vectors are paired with suitable hosts.

A wide variety of suitable vectors for expression in eukaryotic cells are available. Such vectors include pCMVLacI, pXTI (Stratagene Cloning Systems, La Jolla, CA); pCDNA series, pREP series, pEBVHis (Invitrogen, Carlsbad, CA). In certain embodiments, telomerase gene is cloned into a gene targeting vector, such as pMC1neo, a pOG series vector (Stratagene).

Telomerase protein is isolated by standard methods, such as affinity chromatography, size exclusion chromatography, metal ion chromatography, ionic exchange chromatography, HPLC, and other known protein isolation methods. (*see generally* Ausubel et al., *supra*; Sambrook et al., *supra*). An isolated purified protein gives a single band on SDS-PAGE when stained with Coomassie blue.

In one embodiment, the telomerase protein is expressed as a hexa-his fusion protein and isolated by metal-containing chromatography, such as nickel-coupled beads. Briefly, a sequence encoding His₆ is linked to a DNA sequence encoding a telomerase. Although the His₆ sequence can be positioned anywhere in the molecule, preferably it is linked at the 3' end immediately preceding the termination codon. The His-hTI fusion may be constructed by any of a variety of methods. A convenient method is amplification of the TEL gene using a downstream primer that contains the codons for His₆.

F. Peptides and proteins of telomerase

In one aspect of the present invention, peptides having telomerase sequence are provided. Peptides may be used as immunogens to raise antibodies, as inhibitors or enhancers of telomerase function, in assays described herein and the like. Peptides are generally five to 100 amino acids long, and more usually 10 to 50 amino acids. Peptides are readily chemically synthesized in an automated fashion (PerkinElmer ABI Peptide Synthesizer) or may be obtained commercially. Peptides may be further purified by a variety of methods, including high-performance liquid chromatography. Furthermore, peptides and proteins may contain amino acids other than the 20 naturally occurring amino acids or may contain derivatives and modification of the amino acids.

Peptides of particular interest within the context of this invention have the sequence of the intron sequences (Figure 10), the RTase motifs, and the like. In certain embodiments, telomerase proteins have the amino acid sequences presented in Figures 1 or 11, or a portion thereof which is at least 8 amino acids in length (and may be 10, 15, 20 or more amino acids in length). In other embodiments, the protein has one or more amino acid substitutions, additions, deletions. In yet other embodiments, the protein has an amino acid sequence determined by a nucleic acid sequence that hybridizes under normal stringency conditions to the complement of any of the sequences in Figure 11. As indicated above, variants of telomerase include allelic variants.

II. TELOMERASE ASSAYS

A variety of assays are available to determine telomerase activity and expression. Such assays include *in vitro* assays that measure the ability of telomerase to extend a telomeric DNA substrate, nucleolytic activity, primer (telomere) binding activity, dNTP binding activity, telomerase RNA (hTR) binding activity, *in vivo* gain-of-function assays, *in vivo* loss-of function assays, in situ hybridization, RNase probe protection, Northern analysis, amplification of cDNA, antibody staining, and the like.

A. Assays for catalytic activity

Various assays for catalytic activity are described in U.S. Patent Nos. 5,629,154; 5,639,613; 5,645,986 among others. In one conventional assay for telomerase activity, a single-stranded DNA primer having the sequence of the host telomere (e.g., [TTAGGG]_n) and the telomerase enzyme are used (see Shay et al., *Methods in Molecular Genetics* 5:263, 1994; Greider and Blackburn, *Cell* 43:405, 1985; Morin, *Cell* 59:521, 1989; U.S. Patent No. 5,629,154). A preferred assay incorporates a detergent-based extraction with an amplification-based assay. This assay, called TRAP (telomeric repeats amplification protocol), has improved sensitivity (Kim et al., *Science* 266: 2011, 1994). Briefly, in TRAP, telomerase synthesizes extension products, which then serve as templates for amplification. The telomerase products are amplified with a primer derived from a non-telomeric region of the oligonucleotide and a primer derived from the telomeric region. When the amplification products are analyzed, such as by gel electrophoresis, a ladder of products is observed when telomerase activity is present. Permutations of this assay have been described (Krupp et al., *Nucl. Acids Res.* 25: 919, 1997; Savoytsky et al., *Nucl. Acids Res.* 24: 1175, 1996). As well, other telomerase assays are available (Faraoni et al., *J. Chemother* 8: 394, 1996, describing an *in vitro* chemosensitivity assay; Tatematsu et al., *Oncogene* 13: 2265, 1996, describing a "stretch PCR assay"; Lin and Zakian, *Cell* 81: 1127, 1995, describing an *in vitro* assay for *Saccharomyces*).

In addition, catalytic or other activities may be measured by an *in vitro* reconstitution system (see Examples). Briefly, the assays, such as those described herein, are performed using purified telomerase protein that is produced by recombinant

meant and other necessary components, such as the telomerase RNA component, other proteins such as described in WO 98/14593.

B. Assays for other activities

Nucleolytic activity may be assessed by protocols described for example in Collins and Grieder, *Genes and Development* 7: 1364, 1993). The nucleolytic activity is excision of a nucleotide (G from the telomeric repeat TTAGG) from the 3' end of a nucleotide sequence that is positioned at the 5' boundary of the DNA template. Briefly, the activity can be measured by a reaction that uses a nucleic acid template with a 3' nucleotide that is blocking, *i.e.* cannot serve as a primer for a polymerase, unless removed by nucleolytic activity.

Telomere binding activity and assays are described in for example Harrington et al., *J. Biol. Chem.* 270: 8893, 1995. In general, any assay such as a gel-shift assay, that detects protein-nucleic acid interactions may be used. DNTP and RNA binding activity assays are described in Morin, *Eur. J. Cancer* 33: 750 for example.

C. Gain and loss of function

In vivo gain-of-function assays may be performed by transfecting an expression vector encoding telomerase into cells that have no or little detectable endogenous activity. Activity is then measured by an *in vitro* assay, such as those described herein. Another gain of function assay can be performed in tumor cells or other cells expressing telomerase or reverse transcriptase. A telomerase gene is transfected into the cells, expressed at high levels, and these cells are treated with inhibitors of reverse transcriptase. Telomerase activity is then observed as decreased sensitivity to such inhibitors. Furthermore, rescue of function in the yeast telomerase mutant EST2 may be measured.

Loss of function may be measured in cells expressing high levels of telomerase activity, such as LIM 1215 cells or other tumor cells. In this assay, anti-sense oligonucleotide molecules are introduced into the cells, generally in an expression vector. Telomerase gene is verified by diminished telomerase activity. In another

assay, antibodies to telomerase that inhibit function can be used to demonstrate a functional molecule.

D. Expression of telomerase

Expression of telomerase in various cells may be assayed by standard assays using the sequences provided herein. For example, in situ hybridization with radioactive or fluorescent-labeled probes (fragments or oligonucleotides) may be used on tissue sections or fixed cells. Alternatively, RNA may be isolated from the cells and used in Northern, RNase probe protection assays, and the like. Probes for particular regions and probes that are variant specific will generate expression profiles of the various telomerase transcripts.

In a preferred embodiment, telomerase expression is assayed by amplification. Primer pairs for telomerase, including primer pairs for particular variants, are used to amplify cDNA synthesized from cellular RNA. The cDNA may be synthesized from either total RNA or poly(A)+ RNA. Methods and protocols for RNA isolation are well known. The cDNA may be initiated by an oligo(dT) primer, random primers (e.g., dN₆), telomerase specific primer, and the like. The choice of a primer will depend at least in part on the quantity of RNA and the purpose of the assay. Amplification primers are designed to amplify any one of, particular combinations, or all of the variants present in vertebrate cells. Conditions for amplification are chosen to be commensurate with the primer length, base content, length of amplified product and the like. Various amplification systems are available (see Lee et al., *Nucleic Acid Amplification Technologies*, BioTechniques Books, Eaton Publishing, Natick, MA, 1997; Larrick, *The PCR Technique: Quantitative PCR*, BioTechniques Books, Eaton Publishing, Natick, MA, 1997).

Other assays for measuring expression qualitatively and quantitatively are well known. RNase probe protection and Northern analysis are amenable when the amount of telomerase mRNA is sufficient. When very few cells are available, a single cell analysis is desirable, or when the fraction of telomerase RNA in the sample is very low, an amplification protocol is preferred. RNase probe protection, in particular, is well suited for detecting splice variants, mutations, as well as quantitating these RNAs.

As discussed above, in preferred embodiments, expression of the various RNA species is monitored. The different species may be assayed by any method which distinguishes one of the species over the others. Thus, length determination by Northern, RNase probe protection, cloning and amplification are some of the available methods. In preferred embodiments, RNase probe protection and amplification are used. For RNase probe protection, the probe will generally be a fragment derived from the junction of the reference sequence and the intron sequence or derived from the sequence surrounding the intron insertion site. For example, a fragment of the reference telomerase that spans nucleotide 1950-1951 (e.g., nucleotides 1910-1980) will protect the reference sequence as a 71 base fragment, but will protect a telomerase with intron 1 as two fragments of 41 and 30 bases. In contrast, a fragment that contains nucleotides 1910-1950 and 30 bases of intron 1 will protect an intron 1 variant as a 71 base fragment and the reference telomerase as a 41 base fragment. Fragments for RNase probe protection are chosen usually in the range of 30 to 400 bases and are positioned to yield readily distinguishable protection products.

Another method that can be used to distinguish variants is amplification. Amplification primer design and strategy are described above. Briefly, primers that will individually amplify each spliced-in or spliced-out variant are preferred. Multiple reactions can be performed to identify variants with more than one splice-in or splice-out event.

Methods that measure telomerase protein are also useful within the context of the present invention. By way of example, antibodies to telomerase may be used to stain tissue sections or permeabilized cells. Antibodies may also be used to detect protein by immunoprecipitation, Western blot and the like. Furthermore, subcellular localization of telomerase and telomerase variants may be determined using the antibodies described herein.

E. Antibodies to telomerase

Antibodies to the telomerase proteins, fragments, or peptides discussed herein may readily be prepared. Such antibodies may specifically recognize wild type telomerase protein and not a mutant (or variant) protein, mutant (or variant) telomerase

protein and not wild type protein, or equally recognize both the mutant (or variant) and wild-type forms. Antibodies may be used for isolation of the protein, inhibiting (antagonist) activity of the protein, or enhancing (agonist) activity of the protein. As well, assays for small molecules that interact with telomerase will be facilitated by the development of antibodies.

Within the context of the present invention, antibodies are understood to include monoclonal antibodies, polyclonal antibodies, anti-idiotypic antibodies, antibody fragments (e.g., Fab. and F(ab')₂, F_v variable regions, or complementarity determining regions). Antibodies are generally accepted as specific against telomerase protein if they bind with a K_d of greater than or equal to 10⁻⁷M, preferably greater than or equal to 10⁻⁸M. The affinity of a monoclonal antibody or binding partner can be readily determined by one of ordinary skill in the art (see Scatchard, *Ann. N.Y. Acad. Sci.* 51:660-672, 1949).

Briefly, a polyclonal antibody preparation may be readily generated in a variety of warm-blooded animals such as rabbits, mice, or rats. Typically, an animal is immunized with telomerase protein or peptide thereof, which is preferably conjugated to a carrier protein, such as keyhole limpet hemocyanin. Routes of administration include intraperitoneal, intramuscular, intraocular, or subcutaneous injections, usually in an adjuvant (e.g., Freund's complete or incomplete adjuvant). Particularly preferred polyclonal antisera demonstrate binding in an assay that is at least three times greater than background.

Monoclonal antibodies may also be readily generated from hybridoma cell lines using conventional techniques (see U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993; see also *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988). Briefly, within one embodiment, a subject animal such as a rat or mouse is injected with telomerase or a portion thereof. The protein may be administered as an emulsion in an adjuvant such as Freund's complete or incomplete adjuvant in order to increase the immune response. Between one and three weeks after the initial immunization the animal is generally boosted and may tested for reactivity to the protein utilizing well-known assays. The spleen and/or

lymph nodes are harvested and immortalized. Various immortalization techniques, such as mediated by Epstein-Barr virus or fusion to produce a hybridoma, may be used. In a preferred embodiment, immortalization occurs by fusion with a suitable myeloma cell line to create a hybridoma that secretes monoclonal antibody. Suitable myeloma lines include, for example, NS-1 (ATCC No. TIB 18), and P3X63 - Ag 8.653 (ATCC No. CRL 1580). The preferred fusion partners do not express endogenous antibody genes. Following fusion, the cells are cultured in medium containing a reagent that selectively allows for the growth of fused spleen and myeloma cells such as HAT (hypoxanthine, aminopterin, and thymidine). After about seven days, the hybridomas may be screened for the presence of antibodies that are reactive against a telomerase protein. A wide variety of assays may be utilized, including for example countercurrent immuno-electrophoresis, radioimmunoassays, radioimmunoprecipitations, enzyme-linked immuno-sorbent assays (ELISA), dot blot assays, western blots, immunoprecipitation, inhibition or competition assays, and sandwich assays (see U.S. Patent Nos. 4,376,110 and 4,486,530; see also *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988).

Other techniques may also be utilized to construct monoclonal antibodies (see Huse et al., *Science* 246:1275-1281, 1989; Sastry et al., *Proc. Natl. Acad. Sci. USA* 86:5728-5732, 1989; Altling-Mees et al., *Strategies in Molecular Biology* 3:1-9, 1990; describing recombinant techniques). Briefly, mRNA is isolated from a B cell population and utilized to create heavy and light chain immunoglobulin cDNA expression libraries in suitable vectors, such as λ ImmunoZap(H) and λ ImmunoZap(L). These vectors may be screened individually or co-expressed to form Fab fragments or antibodies (see Huse et al., *supra*; Sastry et al., *supra*). Positive plaques may subsequently be converted to a non-lytic plasmid that allows high level expression of monoclonal antibody fragments from *E. coli*.

Similarly, portions or fragments, such as Fab and Fv fragments, of antibodies may also be constructed utilizing conventional enzymatic digestion or recombinant DNA techniques to yield isolated variable regions of an antibody. Within one embodiment, the genes which encode the variable region from a hybridoma

producing a monoclonal antibody of interest are amplified using nucleotide primers for the variable region. These primers may be synthesized by one of ordinary skill in the art, or may be purchased from commercially available sources (e.g., Stratagene, La Jolla, CA). Amplification products are inserted into vectors such as ImmunoZAP™ H or ImmunoZAP™ L (Stratagene), which are then introduced into *E. coli*, yeast, or mammalian-based systems for expression. Utilizing these techniques, large amounts of a single-chain protein containing a fusion of the V_H and V_L domains may be produced (see Bird et al., *Science* 242:423-426, 1988). In addition, techniques may be utilized to change a "murine" antibody to a "human" antibody, without altering the binding specificity of the antibody.

Once suitable antibodies have been obtained, they may be isolated or purified by many techniques well known to those of ordinary skill in the art (see *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988). Suitable techniques include peptide or protein affinity columns, HPLC or RP-HPLC, purification on protein A or protein G columns, or any combination of these techniques.

F. Proteins that interact with telomerase

Proteins that directly interact with telomerase can be detected by an assay such as a yeast 2-hybrid binding system. Briefly, in a two-hybrid system, a fusion of a DNA-binding domain-telomerase protein (e.g., GAL4-telomerase fusion) is constructed and transfected into a cell containing a GAL4 binding site linked to a selectable marker gene. The whole telomerase protein or subregions of telomerase may be used. A library of cDNAs fused to the GAL4 activation domain is also constructed and co-transfected. When the cDNA in the cDNA-GAL4 activation domain fusion encodes a protein that interacts with telomerase, the selectable marker is expressed. Cells containing the cDNA are then grown, the construct isolated and characterized. Other assays may also be used to identify interacting proteins. Such assays include ELISA, Western blotting, co-immunoprecipitations and the like.

III. INHIBITORS AND ENHANCERS OF TELOMERASE ACTIVITY

Candidate inhibitors and enhancers (collectively referred to as "effectors") may be isolated or procured from a variety of sources, such as bacteria, fungi, plants, parasites, libraries of chemicals (e.g., combinatorial libraries), random peptides or the like. Effectors may also be peptides or variant peptides of telomerase, variants of telomerase, antisense nucleic acids, antibodies to telomerase, inhibitors of promoter activity of telomerase, and the like. Inhibitors and enhancers may be also be rationally designed, based on the protein structure determined from X-ray crystallography (see, Livnah et al., *Science* 273:464, 1996). In certain preferred embodiments, the inhibitor targets a specific telomerase, such as a variant.

An inhibitor may act by preventing binding of telomerase to other components of the ribonucleoprotein complex or to the telomere, by causing dissociation of the bound proteins, or by other mechanism. An inhibitor may act directly or indirectly. In preferred embodiments, inhibitors interfere in the binding of the telomerase protein to either the telomerase RNA or to the telomeres. In other preferred embodiments, the inhibitors are small molecules. In a most preferred embodiment, the inhibitors cause a cell to cease replication. Inhibitors should have a minimum of side effects and are preferably non-toxic. Inhibitors that can penetrate cells are preferred.

In other preferred embodiments, an effector is a protein or peptide of telomerase that acts in a dominant negative fashion (see, Ball et al., *Current Biology* 7:71, 1997; *Current Biology* 6:84, 1996). For example, a peptide of telomerase that competitively inhibits the binding of telomerase to telomeres will disrupt the lengthening of telomeres. Generally, these peptides have native sequence, but variants may have increased activity (see, Ball et al., *supra*). Variants may be constructed by the methods described herein. Other peptides may bind telomerase and inhibit one or more of its activities, but do not have telomerase amino acid sequence. Such peptides may be identified by the assays described herein. The proteins or peptides may also increase telomerase activity. For effective inhibition, peptide inhibitors are preferably expressed from vectors transfected or infected into host cells, but may also be introduced by other means, such as liposome-mediated fusion, and the like. Eukaryotic vectors are well

known and readily available. Vectors include plasmids, viral-based vectors, and the like.

In another preferred embodiment, the inhibitor is a ribozyme. "Ribozyme" refers to a nucleic acid molecule which is capable of cleaving a telomerase nucleic acid sequence. Ribozymes may be composed of DNA, RNA, nucleic acid analogues, or any combination of these (e.g., DNA/RNA hybrids). A "ribozyme gene" refers to a nucleic acid molecule which, when transcribed into RNA, yields the ribozyme, and a "ribozyme vector" refers to an assembly that is capable of transcribing a ribozyme gene of interest, and may be composed of either DNA or RNA. Within certain embodiments of the invention, the vector may include one or more restriction site(s) and selectable marker(s). Furthermore, depending on the choice of vector and host cell, additional elements such as an origin of replication, polyadenylation site, and enhancers may be included in the vectors described herein.

As noted above, the present invention also provides ribozymes having the ability to inhibit expression of the telomerase gene. Briefly, a wide variety of ribozymes may be generated for use within the present invention, including for example, hairpin ribozymes (see e.g., Hampel et al., *Nucl. Acids Res.* 18:299-304, 1990, EPO 360,257, and U.S. Patent No. 5,254,678), hammerhead ribozymes (see e.g., Rossi, J.J. et al., *Pharmac. Ther.* 50:245-254, 1991; Forster and Symons, *Cell* 48:211-220, 1987; Haseloff and Gerlach, *Nature* 328:596-600, 1988; Walbot and Bruening, *Nature* 334:196, 1988; Haseloff and Gerlach, *Nature* 334:585, 1988; Haseloff et al., U.S. Patent No. 5,254,678), hepatitis delta virus ribozymes (see, e.g., Perrotta and Been, *Biochem.* 31:16, 1992), Group I intron ribozymes such as those based upon the *Tetrahymena* ribosomal RNA (see, e.g., Cech et al., U.S. Patent No. 4,987,071) RNase P ribozymes (see, e.g., Takada et al., *Cell* 35:849, 1983); as well as a variety of other nucleic acid structures with the capability to cleave a desired or selected target sequence (see e.g., WO 95/29241, and WO 95/31551). Within certain embodiments of the invention, the ribozymes may be altered from their traditional structure in order to include tetraloops or other structures that increase stability (see, e.g., Anderson et al., *Nucl. Acids Res.* 22:1096-1100, 1994; Cheong et al., *Nature* 346:680-682, 1990), or which make the

ribozyme resistant to RNase or endonuclease activity (*see e.g.*, Rossi et al., *Pharmac. Ther.* 50:245-254, 1991).

Within one embodiment of the invention, hairpin and hammerhead ribozymes are provided with the capability of cleaving telomerase nucleic acid sequences. Briefly, hairpin ribozymes are generated so that they recognize the target sequence N₃XN*GUC(N₃₋₆), wherein N is G, U, C, or A, X is G, C, or U, and * is the cleavage site. Similarly, hammerhead ribozymes are generated so that they recognize the sequence NUX, wherein N is G, U, C, or A. The additional nucleotides of the hammerhead ribozyme or hairpin ribozyme is determined by the target flanking nucleotides and the hammerhead consensus sequence (*see* Ruffner et al., *Biochemistry* 29:10695-10702, 1990). The preparation and use of certain ribozymes is described in Cech et al. (U.S. Patent No. 4,987,071). The ribozymes are preferably expressed from a vector introduced into the host cells.

Ribozymes of the present invention, as well as DNA encoding such ribozymes can be readily generated utilizing published protocols (*e.g.*, Promega, Madison Wis., Heidenreich et al., *J. FASEB* 70:90-6, 1993; Sproat, *Curr. Opin. Biotechnol.* 4:20-28, 1993). Alternatively, ribozymes may be generated from a DNA or cDNA molecule which encodes a ribozyme and which is operably linked to a RNA polymerase promoter (*e.g.*, SP6 or T7). An RNA ribozyme is generated upon transcription of the DNA or cDNA molecule.

In other preferred embodiments, inhibitors diminish promoter activity of telomerase. A eukaryotic promoter comprises sequences bound by RNA polymerase and other proteins participating in control of the transcription unit. Telomerase transcription appears to be highly regulated; the protein is expressed mainly in stem, embryonic, and cancer cells, and expressed at much lower levels, if at all, in most somatic cells. Thus, the promoter is a potential target for inhibitors. The inhibitors may disrupt or prevent binding of one or more of the factors that control transcription of telomerase, causing transcription to diminish or cease. The levels of transcription need only fall to a low enough level that at least one telomere becomes absent.

Another inhibitor of the present invention is antisense RNA or DNA to telomerase coding or non-coding sequence. Antisense nucleic acids directed to a particular mRNA molecule have been shown to inhibit protein expression of the encoded protein. Based upon the telomerase sequences presented herein, an antisense sequence is designed and preferably inserted into a vector suitable for transfection into host cells and expression of the antisense. The antisense may bind to any part of the hTI RNA. In certain embodiments, the antisense is designed to bind specifically to one or more variants. Specific binding means that under physiological conditions, the antisense binds to RNAs that have the complementary sequence, but not other RNAs. Because telomerase RNAs that contain any particular intron sequence may be a heterogeneous group of variants due to independent assortment of splice variants, more than one species of RNA may be bound and inactivated. The antisense polynucleotides herein are at least 7 nucleotides long and generally not longer than 100 to 200 bases, and are more typically at least 10 to 50 bases long. Considerations for design of antisense molecules and means for introduction into cells are found in U.S. Patent Nos. 5,681,747; 5,734,033; 5,767,102; 5,756,476; 5,749,847; 5,747,470; 5,744,362; 5,716,846).

In addition, enhancers of telomerase activity or expression are desirable in certain circumstances. At times, increasing the proliferation potential of cells will have a therapeutic effect. For example, organ regeneration or differentiation after injury or diseases, nerve cell or brain cell growth following injury, proliferation of hematopoietic stem cells used in bone marrow transplantation or other organ stem cells, and the like may be limiting and thus benefit from an enhancer of telomerase. Enhancers may stabilize endogenous protein, increase transcription or translation, or act through other mechanisms. As is apparent to one skilled in the art, many of the guidelines presented above apply to the design of enhancers as well.

Screening assays for inhibitors and enhancers will vary according to the type of inhibitor and nature of the activity that is being inhibited. Assays include the TRAP assay or variation, a non-amplification based polymerase assay, yeast two-hybrid, release of repression in yeast transfected with a vertebrate telomerase, and the

like. For screening compounds that interact with the promoter for telomerase, a reporter gene driven assay is convenient.

IV. USES FOR TELOMERASE

Nucleotide sequence for telomerase and telomerase protein are used in a variety of contexts in this invention. In preferred embodiments, the compositions of the present invention are used either as diagnostic reagents or as therapeutics.

A. Diagnostics

Expression of mRNA encoding telomerase and/or protein may be used for detection of dividing cells, especially tumor cells and stem cells. Detection methods include antibody staining or tagged telomerase binding compounds for detection of protein, nucleic acid hybridization in situ for mRNA, hybridization on DNA "chips", Northern analysis, RNase probe protection, amplification by PCR or other method, ligase-mediated amplification and the like. Furthermore, expression of RNA splice variants may be assayed conveniently by amplification, RNase probe protection, other disclosed methods and the like. In particular, oligonucleotide primers surrounding the site of frequent splice variants, such as the primers described herein (e.g., Htel Intron T and HT 2482R) may be used to detect splice variants in various cell types. As shown in the examples, various tumor cell types exhibit different RNA splice variations. Correlation of the splice variant pattern with tumor stage, metastasis potential and the like may be determined. As such, assays for the particular variants may be used as a diagnostic. Cells with increased telomerase activity, such as cancer cells or hyperproliferative cells, may be identified by assaying qualitatively or quantitatively by any of the assays described herein. Typically, telomerase activity or expression will be compared between suspect cells and normal counterpart cells from the same or different individual. Increased activity indicative of a tumor or excessive proliferation is established by direct comparison or by detecting activity in cells otherwise known to be absent in telomerase activity or expression. In addition, monitoring cancer progression or response to therapy can be performed using the assays described herein and comparing activity or expression over a time course.

The variant detected in one ALT cell line, which expresses telomerase, suggests that the basic domain of hT1 may contribute to the ALT mechanism in at least some ALT cell lines. One possible mechanism of ALT could involve dysregulated telomerase components that are inactive in the TRAP assay. Thus, identification of the

5 variants may be useful for following tumorigenesis.

Alternative mRNA splicing is a common mechanism for regulating gene expression in higher eukaryotes and there are many examples of tissue-specific, development-specific and sex-specific alterations in splicing events. Importantly, 15% of mutations linked to disease states in mammals affect splicing patterns (Horowitz and

10 Krainer *Trends Genet.*, 10, 100-106, 1994). Changes in cell physiology can also induce altered splicing patterns. Indeed, tumorigenesis itself has been suggested to enhance the expression of mRNA spliced variants by compromising the alternative splicing mechanisms. Although other, novel minor alternatively spliced hT1 variants may play a role in tumor development, the altered relative expression levels of the major

15 transcripts found in various tumors compared to normal cells, and in post-crisis cell lines compared to limited life-span pre-crisis cells, are likely to play a major role in the establishment and progression of cancers. In addition, the existence of the alternative spliced variants of hT1 that are seen in both testis and colonic crypt, as well as tumor cell lines, suggests complex regulation of this gene in normal development.

Expression of the major hT1 products is found in most tumors and in all

20 telomerase-positive immortalized cell lines. Transcriptional control of hT1 may therefore be a major aspect of the regulation of telomerase activity, in addition to other functions. For example, telomerase may be involved in the healing of chromosome breaks in addition to its role in maintaining telomere length in the germline. The

25 composition of telomerase may vary according to these functional roles.

Therefore, the intron sequences may be especially useful for diagnostic applications. For example, detection and identification of diseases, such as cancer, aging, wound healing, neuronal regeneration, regenerative cells (e.g., stem cells), may be important preludes to determining effective therapy. In this regard, detection of

30 wound healing can facilitate development and identification of an ameliorative

compound. Currently, wound healing assays are expensive and time consuming, whereas an amplification or hybridization-based assay would be quick and cost effective. In any of these applications, detection may be quantitative or qualitative. In a qualitative assay, a particular amplification primer pair or hybridization probe for one of the variant sequences (*e.g.*, introns that are variably spliced) can be used to detect the presence or absence of the variant sequence.

Probes useful in the context of the present invention include nucleic acid molecules that hybridize to the sequences presented in Figure 10 or to their complements. Probes for hybridization are generally at least 24 bases, but may range from 12 to full-length sequence. The probes may comprise additional sequence that does not hybridize to hT1 DNA or RNA. Probes are generally DNA, but may be RNA, PNA, or derivatives thereof. Hybridization conditions will be chosen appropriate for the length of the probe and method of hybridization (*e.g.*, on nylon support, on silicon-based chip). Conditions are well known in the art. One of the sequences in Figure 10 is a genomic sequence, not found in telomerase mRNA. A probe derived from this sequence may be used to detect genomic DNA in RNA preparations and amplification reactions. Hybridization probes may be labeled with a radiolabel, chemiluminescent label, or any of the myriad other known labels.

Hybridization can be performed on mRNA preparations, cDNA preparations, affixed to a solid support, in solution, or in situ tissues, and the like. One type of hybridization analysis is annealing to oligonucleotides immobilized on a solid substrate, such as a functionalized glass slide or silicon chip. Such chips may be commercially procured or made according to methods and procedures set out in *e.g.*, PCT/US94/12282; U.S. Patent No. 5,405,783; U.S. Patent No. 5,412,087; U.S. Patent No. 5,424,186; U.S. Patent No. 5,436,327; U.S. Patent No. 5,429,807; U.S. Patent No. 5,510,270; WO 95/35505; U.S. Patent No. 5,474,796. Oligonucleotides are generally arranged in an array form, such that the position of each oligonucleotide sequence can be determined.

For amplification assays, primer pairs that either flank the introns or require the presence of the intron for amplification are desirable. Many such primer

pairs are disclosed herein. Others may be designed from the sequences presented herein. Generally, the primer pairs are designed to only allow amplification of a single intron, however, in some circumstances detection of multiple introns in the same RNA preparation may be preferred.

5 Other diagnostic assays, such as in situ hybridization, RNase protection, and the like may be used alternatively or in addition to the assays discussed above. The principles that guide these assays are provided by the present invention, while the techniques are well known.

Transgenic mice and mice that are null mutants (*e.g.*, "knockout mice")
10 may be constructed to facilitate testing of candidate inhibitors. The telomerase gene is preferably under control of a tissue-specific promoter for transgenic mice vector constructs. Mice that overexpress telomerase can be used as a model system for testing inhibitors. In these mice, cells overexpressing telomerase are expected to be continuously proliferating. Administration of candidate inhibitors is followed by
15 observation and measurement of cell growth. Inhibitors that slow or diminish growth are candidate therapeutic agents.

Telomerase may also be transfected into cells to immortalize various cell types. Transient immortalization may be achieved by non-stable transfection of an expression vector containing telomerase. Alternatively, proliferation of stable
20 transformants of telomerase gene under control of an inducible promoter can be turned on and off by the addition and absence of the inducer. Similarly, the presence and absence of an inhibitor of telomerase activity may be used to selectively immortalize cells. Expression of part of all of the protein in yeast may act as a dominant negative, as many human proteins interact with components of a complex in yeast, but do so
25 imperfectly and therefore unproductively. As such, these genes act as dominant negatives. Thus, the yeast will eventually senesce. Such cells may be used in screens for inhibitory drugs, which will allow growth of yeast past the time of senescence.

Purified telomerase protein, reference variant protein, or fragments, may be used in assays to screen for inhibitory drugs. These assays will typically be
30 performed *in vitro* and utilize any of the methods described above or that are known in

the art. The protein may also be crystallized and subjected to X-ray analysis to determine its 3-dimensional structure.

B. Therapeutics

5 The compositions and methods disclosed herein may also be used as therapeutics in the treatment of diseases and disorders to effect any of the telomerase activities in a cell. Treatment means any amelioration of the disease or disorder, such as alleviating symptoms of the disease or disorder, reduction of tumor cell mass and the like. For example, inhibitors of enzyme activity may be used to restrict proliferation of cells.

10 Many diseases and disorders are tightly associated with proliferation and proliferative potential. One of the most apparent diseases involving unwanted proliferation is cancer. The methods and compositions described herein may be used to treat cancers, such as melanomas, other skin cancers, neuroblastomas, breast carcinomas, colon carcinomas, leukemias, lymphomas, osteosarcomas, and the like.

15 Other diseases and disorders amenable for treatment within the context of the present invention include those of excessive cell proliferation (increased proliferation rate over normal counterpart cells from the same or different individual) such as smooth muscle cell hyperplasias, skin growths, and the like. Yet other diseases and disorders would benefit from increased telomerase activity. Enhancers of telomerase may be used to

20 stimulate stem cell proliferation and possibly differentiation. As such, expansion of hematopoietic stem cells could be administered in the bone marrow transplant context. As well, many tissues have stem cells. Proliferation of these cells may be beneficial for wound healing, hair growth, treatment of diseases, such as Wilm's tumor, and the like.

Certain of the inhibitors or enhancers may be administered by way of an

25 expression vector. Many techniques for introduction of nucleic acids into cells are known. Such methods include retroviral vectors and subsequent retrovirus infection, adenoviral or adeno-associated viral vectors and subsequent infection, complexes of nucleic acid with a condensing agent (e.g., poly-lysine), these complexes or viral vectors may be targeted to particular cell types by way of an incorporated ligand. Many

30 ligands specific for tumor cells and other cells are well known in the art.

As noted above, within certain aspects of the present invention, nucleic acids encoding ribozymes, antisense, dominant-negative telomerases, portions of telomerase and the like may be utilized to inhibit telomerase activity by introducing a functional gene to a cell of interest. This may be accomplished by either delivering a synthesized gene to the cell or by delivery of DNA or cDNA capable of *in vivo* transcription of the gene product. More specifically, in order to produce products *in vivo*, a nucleic acid sequence coding for the product is placed under the control of a eukaryotic promoter (e.g., a pol III promoter, CMV or SV40 promoter). Where it is desired to more specifically control transcription, the gene may be placed under the control of a tissue or cell specific promoter (e.g., to target cells in the liver), or an inducible promoter.

A wide variety of vectors may be utilized within the context of the present invention, including for example, plasmids, viruses, retrotransposons and cosmids. Representative examples include adenoviral vectors (e.g., WO 94/26914, WO 93/9191; Yei et al., *Gene Therapy* 1:192-200, 1994; Kolls et al., *PNAS* 91(1):215-219, 1994; Kass-Eisler et al., *PNAS* 90(24):11498-502, 1993; Guzman et al., *Circulation* 88(6):2838-48, 1993; Guzman et al., *Cir. Res.* 73(6):1202-1207, 1993; Zabner et al., *Cell* 75(2):207-216, 1993; Li et al., *Hum Gene Ther.* 4(4):403-409, 1993; Caillaud et al., *Eur. J. Neurosci.* 5(10):1287-1291, 1993), adeno-associated type 1 ("AAV-1") or adeno-associated type 2 ("AAV-2") vectors (see WO 95/13365; Flotte et al., *PNAS* 90(22):10613-10617, 1993), hepatitis delta vectors, live, attenuated delta viruses and herpes viral vectors (e.g., U.S. Patent No. 5,288,641), as well as vectors which are disclosed within U.S. Patent No. 5,166,320. Other representative vectors include retroviral vectors (e.g., EP 0 415 731; WO 90/07936; WO 91/02805; WO 94/03622; WO 93/25698; WO 93/25234; U.S. Patent No. 5,219,740; WO 93/11230; WO 93/10218. For methods and other compositions, see U.S. Patent Nos. 5,756,264; 5,741,486; 5,733,761; 5,707,618; 5,702,384; 5,656,465; 5,547,932; 5,529,774; 5,672,510; 5,399,346, and 5,712,378.)

Within certain aspects of the invention, nucleic acid molecules may be introduced into a host cell utilizing a vehicle, or by various physical methods.

Representative examples of such methods include transformation using calcium phosphate precipitation (Dubensky et al., *PNAS* 81:7529-7533, 1984), direct microinjection of such nucleic acid molecules into intact target cells (Acsadi et al., *Nature* 352:815-818, 1991), and electroporation whereby cells suspended in a conducting solution are subjected to an intense electric field in order to transiently polarize the membrane, allowing entry of the nucleic acid molecules. Other procedures include the use of nucleic acid molecules linked to an inactive adenovirus (Cotton et al., *PNAS* 89:6094, 1990), lipofection (Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413-7417, 1989), microprojectile bombardment (Williams et al., *PNAS* 88:2726-2730, 1991), polycation compounds such as polylysine, receptor specific ligands, liposomes entrapping the nucleic acid molecules, spheroplast fusion whereby *E. coli* containing the nucleic acid molecules are stripped of their outer cell walls and fused to animal cells using polyethylene glycol, viral transduction, (Cline et al., *Pharmac. Ther.* 29:69, 1985; and Friedmann et al., *Science* 244:1275, 1989), and DNA ligand (Wu et al., *J. of Biol. Chem.* 264:16985-16987, 1989), as well as psoralen inactivated viruses such as Sendai or Adenovirus. In one embodiment, the nucleic acid molecule is introduced into the host cell using a liposome.

Administration of effectors will generally follow established protocols. The compounds of the present invention may be administered either alone, or as a pharmaceutical composition. Briefly, pharmaceutical compositions of the present invention may comprise one or more of the inhibitors or enhancers as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like, carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide) and preservatives. In addition, pharmaceutical compositions of the present invention may also contain one or more additional active ingredients. Effectors may be further coupled with a targeting moiety that binds a cell surface receptor specific to the proliferating cells.

Compositions of the present invention may be formulated for the manner of administration indicated, including for example, for oral, nasal, venous, intracranial, intraperitoneal, subcutaneous, or intramuscular administration. Within other embodiments of the invention, the compositions described herein may be administered as part of a sustained release implant. Within yet other embodiments, compositions of the present invention may be formulated as a lyophilizate, utilizing appropriate excipients which provide stability as a lyophilizate, and subsequent to rehydration.

As noted above, pharmaceutical compositions also are provided by this invention. These compositions contain any of the above described ribozymes, DNA molecules, proteins, chemicals, vectors, or host cells, along with a pharmaceutically or physiologically acceptable carrier, excipients or diluents. Generally, such carriers should be nontoxic to recipients at the dosages and concentrations employed. Ordinarily, the preparation of such compositions entails combining the therapeutic agent with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrans, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents.

In addition, the pharmaceutical compositions of the present invention may be prepared as medicaments for administration by a variety of different routes, including for example intraarticularly, intracranially, intradermally, intrahepatically, intramuscularly, intraocularly, intraperitoneally, intrathecally, intravenously, subcutaneously or even directly into a tumor. In addition, pharmaceutical compositions of the present invention may be placed within containers, along with packaging material which provides instructions regarding the use of such pharmaceutical compositions. Generally, such instructions will include a tangible expression describing the reagent concentration, as well as within certain embodiments, relative amounts of excipient ingredients or diluents (e.g., water, saline or PBS) which may be necessary to reconstitute the pharmaceutical composition. Pharmaceutical compositions are useful for both diagnostic or therapeutic purposes.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease. Dosages may
 5 be determined most accurately during clinical trials. Patients may be monitored for therapeutic effectiveness by appropriate technology, including signs of clinical exacerbation, imaging and the like.

The following examples are offered by way of illustration, and not by
 10 way of limitation.

EXAMPLES

EXAMPLE 1

IDENTIFICATION AND ISOLATION OF THE HUMAN TELOMERASE GENE

5

A human telomerase gene is identified in a cDNA library constructed from a cancer cell line. The cDNA is subjected to DNA sequence analysis (Kilian et al., *supra*).

10 An EST sequence, GenBank Accession No. AA281296, is identified as partial telomerase gene sequence by a BLAST search against the *Euplotes* telomerase sequence, GenBank Accession No. U95964 ($p=3.2 \times 10^{-6}$). Amino acid sequence identity between the two sequences is approximately 38% and amino acid sequence similarity is approximately 60%.

To obtain longer clones of hT1, a number of cDNA libraries prepared
15 from tumor cells are screened by amplification using primers from within the EST sequence. Primers HT1553F and HT1920R, based on the EST sequence, are used to amplify an approximately 350 bp fragment in a variety of cDNA libraries. The amplification reaction is performed under "hot start" conditions. Amplification cycles are 4 min at 95°C; 1 min at 80°C; 30 cycles of 30 sec at 94°C, 30 sec at 55°C, 1 min at
20 72°C; and 5 min at 72°C. An amplified product of the expected size (~350 bp) is detected in only 3 of the 12 libraries screened. No fragment is detectable in a testis cDNA library, somatic cell libraries, and a variety of cancer cell cDNA libraries. However, an abundant 350 bp fragment is detected in a cDNA library from LIM 1215 cells, a colon cancer cell line. In this library, and in several others, an additional
25 fragment of around 170 bp was amplified.

Two approaches are followed to obtain longer clones from the LIM1215 library: screening plaques with a 32 P-labeled EST probe and amplification on library DNA. A single positive plaque, designated 53.2, with a 1.9 kb insert is obtained by hybridization of the library with the EST probe. DNA sequence analysis of this clone
30 demonstrates that it extends both 5' and 3' of the EST sequence, but did not contain a

single open reading frame (ORF). A fragment obtained from amplification analysis of the library is similar in sequence to the 53.2 fragment but also contains two additional sequences of 36bp and >300bp. Both insertions demonstrate characteristics of splice acceptor and donor sequences at their boundaries relative to the 53.2 sequence and may represent unspliced introns. Amplification using primers T7 and HT1553F, yields an approximately 1.6 kb fragment; and using primers T3 and HT1893R, yields an approximately 0.7 kb fragment. Each of these fragments support amplification of a 320 bp fragment using primers HTEL1553F and HT1893R.

Longer clones may also be obtained by amplification of mRNA samples.

Reverse transcriptase PCR (RT-PCR) on LIM1215 mRNA identifies a number of additional PCR products, including one with a 182 bp insertion relative to 53.2 that results in a single open reading frame (ORF). cDNA is synthesized from RNAs isolated from normal and tumor tissues. RT-PCR followed by nested amplification is performed using the Titan RT-PCR system (Boehringer-Mannheim). Amplification conditions are as follows: 95°C for 2 min, two cycles of 94°C for 30 sec, 65°C for 30 sec and 68°C for 3 min, 2 cycles of 94°C for 30 sec, 63°C for 30 sec, 68°C for 3 min, 34 cycles of 94°C for 30 sec, 60°C for 30 sec and 68°C for 3 min. RT-PCR products are diluted 100 fold, and 1 µl is used for nested amplification using *Taq* polymerase with buffer Q (Qiagen). Amplification conditions are as above, except that the final step is 14 cycles. For normal tissues and tumors, amplification products are resolved by electrophoresis in 1.5% agarose gel, transferred to Zetaprobe membrane and probed with radiolabeled oligonucleotide HT1691F.

The DNA sequence is also extended 5' and 3' using a combination of cRACE and 3' RACE, respectively, on LIM1215 mRNA to give a fragment of 3871 bp designated hT1 (Figure 1). Two rounds of cRACE are carried out to extend the sequence of hT1 and map the transcription initiation site. 500 ng LIM1215 polyA+ RNA is used as the template. First strand cDNA synthesis is primed using the HT1576R primer. The first round of amplification on the ligation product (using the XL-PCR system) employs the HT1157R and HT1262F primers. Amplification products are purified using Qiagen columns, and further amplified using primers

HT1114R and HT1553F. A resulting 1.4 kb band is subjected to DNA sequence analysis, and a new set of primers are designed based on this sequence. For the second round of cRACE, the first strand cDNA is primed with the HT220R primer. The first round of amplification utilizes the HT0142R and HT0141F primers. Products are purified as above and amplified using HT0093 and HT0163F primers. A product of 100 bp is observed and subjected to sequence analysis in two independent experiments to define the 5' end of the hT1 transcript. The 5' end of the transcript is also obtained by amplification using primer HtelFulcodT 5'-AGGAGATCTCGCGATGCCGCGCTC-3' and HtelFulcodB 5'-TCCACGCGTCCTGCCCGGGTG-3' on LIM1215 RNA. The resulting amplified product was digested with Mlu I and Bgl II and ligated to the remaining telomerase cDNA sequence.

The 3'-most sequences of the transcript are obtained by two rounds of amplification (XL-PCR system) using EBHT18 in both rounds as the reverse primer, and HT2761F and HT3114F as the forward primers in the first and second rounds, respectively.

The size of hT1 accords well with the size estimated from the Northern blot (see below) for the most abundant RNA species in LIM1215 RNA. Approximately 3.9 kb of DNA sequence is presented in Figure 1. The sequence found in the EST is located from nucleotides 1624-2012. The predicted amino acid sequence of the largest open reading frame is also presented in Figure 1. As presented, the protein is 1132 amino acids.

Table 2

25	Name	Oligo Sequence
	HT0029F	5' - GCTGGTGCAGCGCGGGGACC
	HT 5'Met	5' - CACAAGCTTGAATTCACATCTCACCATGAAGGAGCTGGTGGCCCGAGT
	HT0093R	5' - GGCACGCACACCCAGGCACTG
30	HT0141F	5' - CCTGCCCTGAAGGAGCTGGTG
	HT0142R	5' - GGACACCTGGCAGAGGAG
	HT0163F	5' - CCGAGTGGCTGCAGAGGCTGT
	HT0220R	5' - GAAGCCGAAGGCCAGCACGTTCTT
	HT1262F	5' - GTGCAGCTGCTCCGCCAGCACA
35	HT1114R	5' - GTTCCCAGCAGCTCCAGAAACAG
	HT1157R	5' - GGCAGTGGCTCTTGAGGAGCA
	HT1553F	5' - CACTGGCTGATGAGTGTGATC

	HT1576R	5'	- GACGTACACACTCATCAGCCAG
	HT1590F	5'	- GGTCTTTCTTTTATGTACCGAG
	HT1691F	5'	- CACTTGAAGAGGGTGCAGCT
	HT1875F	5'	- GTCTCACCTCGAGGGTGAAG
5	HT1893R	5'	- TTCACCCCTCGAGGTGAGACGCT
	HT1920R	5'	- TCGTAGTTGAGCACGCTGAAC
	HT2026F	5'	- GCCTGAGCTGTACTTTGTCAA
	HTM2028F	5'	- CTGAGCTGTACTTTGTCAAGGACA
	HT2230F	5'	- GTACATGCCAGAGTTCGTGGCTCA
10	HT2356R	5'	- CATGAAGCGTAGGAAGACGTCGAAGA
	HT2482R	5'	- CGCAAAACAGCTTGTCTCCATGTC
	HT2761F	5'	- CTATGCCCGGACCTCCATCAGA
	HT2781R	5'	- CTGATGGAGGTCCGGGCATAG
	HT3114F	5'	- CCTCCGAGGCCGTGCAGT
15	HT3292B	5'	- CACCTCAAGCTTTCTAGATCAGTCCAGGATGGTCTTGAAGTCA
	HT3689R	5'	- GGAAGGCAAGAGGCGCAGGGCGA
	EBHT18	5'	- CACGAATTCGGATCCAGCTTTTTTTTTTTTTTTTTT
	HT-RNA-F	5'	- GGGTTGCGGAGGGTGGGC
	HT-RNA451R	5'	- GCAGTGGTGAGCCGAGTCCCTG
20	HT-RNA598F	5'	- CGACTTTGAGGTTGCCCTCA
	Htel 5'T	5'	- GCTGGTGCAGCGCGGGGACC
	Htel1979T	5'	- GAGGTGCAGAGCGACTACTCCA
	Htel1.335T	5'	- GTCTCACCTCGAGGGTGAAG
25	Htel171T	5'	- GGCTGCTCTTGGCTTGGTGA
	Htel121B(Top)	5'	- GCCAGAGATGGAGTACCC
	Htel121B(Bot)	5'	- GGGTGGCTCGATCTCTGGC
	Htel-7B	5'	- CCGCACGCTCATCTCTCCAGCT
	Htelr256B	5'	- GCTTGGGGATGAACGGTC
30	HtelIronT	5'	- GCCTGAGCTGTACTTTGTCA
	Htel 3'CODB	5'	- CACCTCAAGCTTTCTAGATCAGTACGGCCCCAGCCCACTCCCCT
	Htel 1210B	5'	- GCAGCACACATGCTGAACCTGT
	Htel 1274B	5'	- GTGTCAAGGATGACGCGCAGGAA
	Htel 1624b	5'	- ACCCACACTTGCCTTGCCTGAGT
35	hTR TAC	5'	- ACTGGATCTCTGACAAATTAATGCATCGGCTCGTATAATGTGTGGAGGGTTGCGGAGGG TGGGC
	hTR 5'T7	5'	- CTGTAAATACGACTCACTATAGGGTTGCGGAGGGTGGGC
	hTR 3'Pst	5'	- CACCTGCGACATGCTTTTCCTCTCACGGACTCATCAGGCCAGCTGGCGACGCATGTGT GAGCCGAGTCTG
40	BT-177	5'	- GGATCCGCGCGAGAGCAGCGTCTG
	BT-178	5'	- CGAAGCTTTCAAGTGGCGGGCATCTGAAC
	BT-179	5'	- CGAAGCTTTCAAGGCGCCAGCCCACTCC
	BT-182	5'	- GCGGATCCAGAGCCAGCTCTACGTC
45	BT-183	5'	- GCGGATCCGCTTCAGATGTCGGGCCAC

EXAMPLE 2

HT1 SEQUENCE AND ALIGNMENT WITH OTHER TELOMERASES

50

Multiple sequence alignment demonstrates that the predicted hT1 protein is co-linear with the *Euplotes* and *S. cerevisiae* telomerase catalytic subunits over their entire lengths (Figure 2). Although the overall homology between the three proteins is

relatively low (approximately 40% similarity in all pairwise combinations) the overall structure of the protein seems to be well conserved. Four major domains: N-terminal, basic, reverse transcriptase (RT) and C-terminal are present in all three proteins. The highest area of sequence similarity is within the RT domain. Notably, all the motifs characteristic of the *Euplotes* RT domain are present and all amino acid residues implicated in RT catalysis are conserved in the hT1 sequence (Lingner et al., *Science* 276: 561-567, 1997).

Recently, protein phosphatase 2A treatment of human breast cancer cell extracts has been shown to inhibit telomerase activity (Li et al., *J. Biol. Chem.* 272: 16729-16732, 1997). It is not known whether this effect is direct, but it raises the possibility of regulation of telomerase activity by protein phosphorylation. The predicted hT1 protein does contain numerous potential phosphorylation sites, including 11 SP or TP dipeptides, which are potential sites for cell cycle dependent kinases.

EXAMPLE 3

CHARACTERIZATION OF TELOMERASE GENE

Northern analysis and Southern analysis are performed to determine the size of the telomerase transcript and whether telomerase gene is amplified in tumors cells.

For Northern analysis, polyA mRNA is isolated from LIM 1215 cells and from CCD fibroblasts. CCD is a primary human fibroblast cell line. Briefly cells are lysed by homogenization in a buffered solution (0.1 M NaCl, 10 mM Tris, pH 7.4, 1 mM EDTA) containing detergent (0.1% SDS) and 200 µg/ml of proteinase K. SDS is added to the lysate to a final concentration of 0.5%, and the lysate is incubated at 60°C for 1 hr and 37°C for 20 min. The lysate is then incubated for 1 hr with a slurry oligo dT-cellulose that has been pre-cycled in 0.1 M NaOH and equilibrated in 0.5 M NaCl, 10 mM Tris pH 7.4, 1 mM EDTA, and 0.1% SDS. The resin is collected by centrifugation, batch washed in the equilibration buffer, and loaded into a column. The

mRNA is eluted with warmed (37°C) buffer (10 mM Tris pH 7.4, 0.1 mM EDTA) and ethanol precipitated.

Approximately 3 µg of polyadenylated RNA is electrophoresed in a 0.85% formaldehyde-agarose gel (see Sambrook et al., *supra*) and transferred overnight to Genescreen plus (Bio-Rad, CA). The membrane is hybridized with a ³²P-labeled telomerase-specific probe (390 bp insert corresponding to the EST sequence). After washing the blot at high stringency, a prominent ~3.8 kb band is observed in mRNA from LIM 1215, but not in mRNA from CCD fibroblasts (Figure 3). Subsequent hybridization of the same membrane with a probe for glyceraldehyde 6-phosphate dehydrogenase demonstrated an equivalently strong band in both mRNAs, indicating that each lane contained a similar amount of high quality RNA. The presence of larger transcripts (especially a ~8 kb heterodispersed band) is also visible only in LIM1215 RNA (Fig. 10, upper panel). These findings provide an indication of additional hT1-specific mRNA and also that hT1 may be preferentially expressed in tumor versus normal cells.

For Southern analysis, DNA is isolated from human peripheral blood mononuclear cells and LIM 1215. Approximately 10 µg of DNAs are digested with *Hind* III, *Xba* I, *Eco* RI, *Bam*HI, and *Pst*I, electrophoresed in a 1% agarose gel, and transferred to a nylon membrane. For controls, plasmid DNA containing human telomerase is titrated to approximately the equivalent of 10 copies, 5 copies, and 1 copy per 10 µg genomic DNA and electrophoresed on the same gel. A 390 bp fragment of telomerase gene (containing the EST sequence) is ³²P-labeled and hybridized under normal stringency conditions. The filter is washed in 2X SSC, 0.1% SDS at 55°C. A scanned phosphor image is presented in Figure 4. As shown, the telomerase gene does not appear to be amplified or rearranged in LIM1215 as there is not significant difference in the pattern or intensity of hybridization when comparing LIM 1215 to PBMC DNA. Moreover, telomerase appears to be a single copy gene, as all digestions except *Pst* I yielded a single band.

EXAMPLE 4

hT1 EXPRESSION PATTERNS

Although telomerase activity has been widely associated with tumor cells and the germline, it has only recently been recognized that certain normal mammalian tissues express low levels of telomerase activity. hT1 expression is not detected in primary fibroblast RNA, and amplification of several commercially available cDNA libraries from lung, heart, liver, pancreas, hippocampus, fetal brain, and testis using primers for the EST region, did not reveal any products.

However, the expression of hT1 in normal tissues that have previously been shown to have telomerase activity (colon, testis and peripheral blood lymphocytes) are examined, as well as a number of melanoma and breast cancer samples. RNA is isolated from normal human colon, testis and circulating lymphocytes, and from tissue sections of tumor samples, and subjected to RT-PCR analysis. Amplification products from cDNA are easily distinguished from products resulting from contaminating genomic DNA, as a product of ~300 bp is observed using cDNA as a template and a product of 2.7 kb is observed using genomic DNA as a template. hT1 transcripts are detected in both colon and testis, in the majority of tumor samples, and very weakly in the lymphocyte RNA (Figure 5, upper panel). Interestingly, two of the breast cancer samples are negative for hT1 expression, despite containing comparable amounts of RNA to the other samples, as judged by amplification of β -actin as a positive control (Figure 5, lower panel).

Acquisition of telomerase activity appears to be an important aspect of the immortalization process. The expression of hT1 in a number of matched pairs of pre-crisis cell cultures and post-crisis cell lines is determined using RT-PCR followed by amplification from nested primers (Figure 6, upper panel). These cell lines are telomerase negative (pre-crisis cell line) and positive (post-crisis cell lines), respectively, using the TRAP assay (Bryan et al., *EMBO J.* 14: 4240-4248, 1995). In two matched pairs, BFT-3B and BET-3K, hT1 is detected only in the post-crisis cell lines (compare lanes a and b, lanes e and f). While the post-crisis line (lanes d, f) in the

BFT-3K set shows an abundant hT1 band, a fragment of the same size is also weakly present in the pre-crisis (lanes c, e) culture sample. In addition, two of the three post-crisis cell lines demonstrate the presence of an additional unexpected fragment of 320 bp, and this product is also observed when colon and testis mRNA are analyzed on high resolution gels.

Three immortalized telomerase-negative (ALT) cell lines are also analyzed for hT1 expression (Figure 6, lanes g, h, i). Two of the lines appear negative for hT1 expression, but in one line (HICF-T/B1), a product of approximately 320 bp is again amplified (Figure 6, lane i), similar to the post-crisis, colon and testis samples.

DNA sequence analysis of the 320 bp product from the line HICF-T/B1 (ALT) reveals the presence of a 38 bp insertion, relative to the expected product. The possibility that this is an amplification from genomic DNA rather than mRNA is ruled out by performing amplification with the same primers but using genomic DNA as the template. Under these conditions, a 2.7 kb fragment is amplified and its authenticity confirmed by partial sequence analysis.

EXAMPLE 5

IDENTIFICATION OF ALTERNATIVE SPLICING PATTERNS OF TELOMERASE mRNA

DNA sequence analysis of clones from the LIM1215 cDNA library and the RT-PCR data presented above for the pre-crisis and post-crisis cultures indicated that there is a number of different sequence variants within the hT1 transcript. To systematically survey for variants, RT-PCR is performed using primer pairs covering the whole sequence. No variants are observed in the N-terminal and the basic domains, but several variants are observed in the RT domain and, to a lesser extent, the C-terminal domain. Most notably, there are several RNA variants between RT Motif A and RT Motif B (Figure 7A).

Samples of mRNA are prepared from several different tumors using conventional protocols. The tumors are: (1) SLL lung carcinoma, (2) Lymphoma C,

(3) Lung carcinoma, (4) Medullablastoma A, (5) Lymphoma B, (6) Lymphoma E, (7) Tumor sample 47D, (8) Pheochromocystoma, (9) Lymphoma F, (10) Glioma, and (11) Lymphoma G. The mRNAs from these samples are first reverse transcribed to cDNAs and then amplified using primers HT1875F and HT2781R, followed by amplification with nested primers HT2026F and HT2482R. Four different amplified products are observed in Figure 8: 220 bp (band 1), 250 bp (band 2), 400 bp (band 3) and 430 bp (band 4). Strikingly, there is considerable variation among the tumor samples tested both in the total number of amplified products and in the quantitative distribution among the products.

Three of these products are isolated from a number of tumor tissues and subjected to DNA sequence analysis. One of them, a 220 bp fragment, is equivalent to the 53.2 cDNA from the LIM1215 library. The fragment of the ~250 bp (band 2) contains a 36 bp in-frame insertion, the same insertion that was identified in an amplified product from a LIM1215 cDNA library. As the RT-PCR product had the same sequence as the product from the cDNA library, it is apparent that the 36 bp insertion is not an artifact generated during library construction. The largest product (band 4) contains a 182 bp insertion (the same as the larger product amplified earlier from LIM1215 RNA) compared to the 250 bp amplicon. Unambiguous sequence for the 400 bp band (band 3) is not obtained. Based on its size, it may contain the 182 bp insert but missing the 36 bp insertion present in bands 2 and 4 and absent from band 1.

To test the hypothesis that such a transcript exists, a primer, HTM2028F, is designed such that amplification ensues only when the 36 bp fragment was missing. Amplification using HTM2028F and HT2026F primers in combination with HT2356R demonstrate that transcripts containing the 182 bp fragment but missing the 36 bp fragment are present in LIM1215 RNA (Figure 9, lanes a and b). The same top strand primers (HTM2028F and HT2026F) in combination with HT2482R primer amplify a number of products from LIM1215 RNA (Figure 9, lanes c and d), most of which represent bands 1- 4 as determined by direct sequence analysis of PCR products. An amplified fragment of 650 bp using HTM2028F and HT2482R primers represents another, not yet fully characterized, alternatively spliced telomerase variant in the RT-

MotifA/RT Motif B region. For clarity of presentation, the protein sequence giving the best match with *Euplotes* and *S. cerevisiae* proteins is presented in Figure 1 as the reference sequence.

- Specifically, there are at least seven inserts or introns that can be present (or absent) from telomerase RNA. (1) The 5'-most sequence (Y) is located between bases 222 and 223. (2) the insert (X) is located between bases 1766 and 1767. A partial sequence is determined and is presented in Figure 10. Termination codons are present in all three reading frames. Thus, a truncated protein without any of the Rtas motifs would be produced. (2) A sequence, indicated as "1" in Figure 7, is located between bases 1950 and 1951. This intronic sequence is 38 bp (Figure 10) and appears to be present in ALT and most tumor lines. The presence of this sequence adds 13 amino acids and shifts the reading frame, such that a termination codon (TGA) is in frame at nucleotide 1973. (3) A sequence, indicated as " α " in Figure 7, is located between bases 2130 and 2167. This sequence is 36 bp (Figure 10) and its absence removes RTase motif "A" but does not alter the reading frame. (4) A sequence, indicated as " β " in Figure 7 is present between bases 2286 and 2469. The insert is 182 bases (Figure 10) and its absence causes a reading frame-shift and a termination codon in RTase motif 5 at nucleotide 2604. (5) The sequence "2" in Figure 7 is present between bases 2823 and 2824. Its length is undetermined; its partial sequence is presented in Figure 10. The presence of this insert causes a truncated telomerase protein, as the first codon of the insert is a termination codon. (6) The sequence "3" is a 159 bp insert (Figure 10) between bases 3157 and 3158. Its presence leads to a telomerase protein with an altered COOH-terminus. The insert contains a stop codon. Moreover, sequence "3" has a putative binding site for the SH3 domain of *c-abl* (PXXXXPXXP; PEMEPPRRP).
- The transcript that most closely aligns with *Euplotes* and yeast telomerases by amino acid similarity contains sequences A and B, and does not contain sequence C. The nucleotide and amino acid sequences of eight variants resulting from mRNAs comprising combinations of sequences A, B, and C are presented in Figure 8.

EXAMPLE 6

RECOMBINANT EXPRESSION OF HUMAN TELOMERASE

Human telomerase is cloned into bacterial expression vectors. The sequence shown in Figure 1 is amplified from LIM 1215 mRNA in two pieces and then ligated together.

For the amplification, first strand cDNA is synthesized and used in an amplification reaction (Titan system, Boehringer, IN) with a mixture of DNA polymerases, such that a proofreading thermostable enzyme (*e.g.*, *rTth*) is used with *Taq* DNA polymerase. As much of the mRNA in LIM 1215 lacks sequence B (Figure 9), the amplification primers are designed such that one primer of each pair is within sequence B, on either side of the *Sac* I site at nucleotide 2271 (Figure 1). The 5' portion is first amplified from cDNA using HT2356R and HT0028F primers (cycle conditions: 70°C, 2 min; then added primer sequences equilibrated to 50°C; 50°C, 30 min; 95°C, 2 min; 2 cycles of 94°C, 30 sec; 65°C, 30 sec; 3 cycles of 94°C, 30 sec; 63°C, 30 sec; 68°C 3 min; 32 cycles of 94°C, 30 sec; 60°C, 30 sec; 68°C, 3 min). The extreme 5' portion of the telomerase gene is then ligated in *Eco* RI/ *Sac* I digested pTTQ18 (Amersham International plc, Buckinghamshire, England) and pBluescriptII KS+, and the sequence verified.

To obtain the 3' end, LIM 1215 cDNA is amplified using HT2230F and a HT3292B primer that is complementary to the sequence encoding the very C-terminus of telomerase. The amplification products are digested with *Hind* III and *Sac* I and inserted into pTTQ18 and pBluescript II KS+. The 5' and 3' ends are also cloned joined at the native *Sac* I site in pTTQ18 both as a Hexa-His fusion and a non-fusion protein.

The plasmid pTTQ18-Htel is transfected into bacterial cells (*e.g.*, BL21(DE3)). Over expression of the protein is accomplished upon induction with IPTG. The bacteria are collected by centrifugation and lysed in lysis buffer (20 mM NaPO₄, pH 7.0, 5 mM EDTA, 5 mM EGTA, 1 mM DTT, 0.5 µg/ml leupeptin, 1 µg/ml aprotinin, 0.7 µg/ml pepstatin). This mixture is evenly suspended via a Polytron homogenizer and the cells are broken open by agitation with glass beads or passage

through a microfluidizer. The resulting lysate is centrifuged at 50,000 rpm for 45 min. The supernatant is diluted with 20 mM NaPO₄, 1 mM EDTA, pH 7.0 (buffer A). The diluted lysate supernatant is then loaded onto a SP-Sepharose or equivalent column, and a linear gradient of 0 to 30% SP Buffer B (1 M NaCl, 20 mM NaPO₄, 1 mM EDTA, pH 7.0) in Buffer A with a total of 6 column volumes is applied. Fractions containing telomerase are combined. Further purifications can be performed.

For hexa-His fusion proteins, the lysate is clarified by centrifugation and batch absorbed on a Ni-IDA-Sepharose column. The matrix is poured into a column and washed with buffer, typically either 50 mM Tris pH 7.6, 1 mM DTT; 50 mM MES pH 7.0, or IMAC buffer (for hexa-his fusions). The telomerase protein bound to the matrix is eluted in NaCl containing buffer.

EXAMPLE 7

15 RECOMBINANT EXPRESSION OF HUMAN TELOMERASE RNA COMPONENT

The human telomerase RNA component is first isolated by amplification from genomic DNA. The amplification primers are telRNA T and telRNA 598B (Figure 5). Amplification conditions are 95°C, 3 min; addition of polymerase; 80°C 2 min; 35 cycles of 94°C, 30 sec; 68°C, 2 min.

The amplified product is inserted into pBluescript after another amplification using hTR TAC (has a tac promoter sequence) and hTR 3'Pst (has a cis-acting ribozyme sequence) primers. The pBluescript insert is then isolated and ligated to pACYC177.

EXAMPLE 8

EXPRESSION OF HUMAN TELOMERASE SUBREGIONS

The RTase domain of human telomerase is determined by sequence comparison with Moloney MuLV reverse transcriptase. The fingers/palm region of Moloney MuLV reverse transcriptase forms a stable unit for crystallization (Georgiadis et al., *Structure* 3: 879, 1995). A number of residues and motifs are conserved in the active site of both proteins. Primers are designed to amplify the RTase domain and the fingers/palm domain for insertion into an expression vector and subsequent protein isolation.

Fragment ID	Primers	Amino acids
I	BT-177 / BT-178	AAEH... → ...VQMPAH
II	BT-177 / BT-179	AAEH... → ...VGLGL
III	BT-182 / BT-179	RATS... → ...VGLGL
IV	BT-183 / BT-179	VQMPAH... → ...VGLGL

Fragment I encodes the "fingers and palm" domain that corresponds to MoMuLV. The C-terminal "thumb" and "connection" (see, Kohlstaedt et al., *Science* 256: 1783, 1992) are deleted. Fragment II encodes the telomerase reverse transcriptase domain, as well as the C-terminal "connection" domain. The N-terminus is chosen by size comparison with the MoMuLV RTase structure. Fragment III encodes the C-terminus of the protein. The RATS sequence is located within the RTase domain (palm region) of the protein. Fragment IV encodes the C-terminal region containing the "thumb" and "connection" domains and may function as a regulatory element. The connection domain in HIV-1 is able to block the catalytic cleft of HIV RTase in the absence of the RNase domain (Kohlstaedt et al, *supra*). In an analogous fashion, the C-terminal region may be useful as a regulatory (inhibitory) fragment. Moreover, sequence C has a putative binding site for the SH3 domain of *c-abl* (PXXXXPXXP; PEMEPPRRP, see variant 2 sequence of Figure 8). *c-abl* protein interacts directly with

the ATM (ataxia telangiectasia) protein (Shafman et al., *Nature* 389: 520, 1997), a protein apparently involved in cell-cycle control, meiotic recombination, telomere length monitoring and DNA damage response. Binding of *c-abl* protein may be assessed in standard protein-protein interaction methods. As such, an interaction of telomerase and *c-abl* or other SH3-domain containing proteins (e.g., *erb2*) and regulation by movement of the telomerase C-terminus in and out of the catalytic cleft may be controllable using the constructs and products described herein. In one instance, regulation may be mediated by phosphorylation/dephosphorylation reactions.

All primers have either a *Hind* III or a *Bam* HI site. The amplification reaction is performed in 1X *Pfu* buffer. 250 μ M dNTPs, 100 ng each primer, clone 53.2 template DNA using the following cycling conditions: 94°C for 2 min; 25 cycles of either 55°C, 60°C, or 65°C for 2 min, 72°C for 2 min, 94°C for 1 min; followed by 72°C for 10 min. Products of the predicted length are obtained (966 bp for BT-177/BT-178; 1479 bp for BT-177/BT-179; 824 bp for BT-182/BT-179; 529 bp for BT-183/BT-179). The amplified products are extracted with phenol:CHCL₃ and precipitated with ethanol. The products are resuspended and digested with the appropriate enzyme that cleaves in the primer sequence.

The digested products are ligated to pBluescript that is digested with enzymes that leave compatible ends. The inserts are digested with *Hind* III and partially digested with *Bam* HI for ligation to pGEX. The plasmid is transfected in BL21(DE3) cells and selected on ampicillin plates. Colonies are picked and grown overnight in liquid broth. An aliquot is diluted in Terrific Broth with 100 μ g/ml ampicillin. The cells are grown at 37°C and induced with 0.5 mM IPTG at approximately O.D. 0.8. Growth is continued for 5 hours. Cells are collected by centrifugation and may be processed immediately or frozen at -70°C until needed.

Protein is purified from lysed cells. Cell pellets are lysed by vortexing in 50 mM Tris pH 8.0, 10 mM 2-ME, 1 mg/ml lysozyme, 0.5% Triton X-100, 1 μ g/ml pepstatin, 10 μ g/ml leupeptin, 10 μ g/ml aprotinin, 0.5 mM PMSF, and 2 mM EDTA and a freeze/thaw cycle. Lysates are clarified by centrifugation. Supernatant is added to a 50% slurry of GSH-Sepharose, rotated at 4°C for 2 hr. The matrix is washed twice

with lysis buffer, followed by 50 mM Tris, pH 8.0, 10 mM 2-ME. For analysis by SDS-PAGE gel electrophoresis, sample buffer with 150 mM 2-ME is added and the samples boiled.

EXAMPLE 9

ISOLATION OF MURINE TELOMERASE GENE

The murine telomerase gene is isolated from genomic or cDNA library.

- 10 A mouse genomic library is constructed in λ FIX II vector from strain 129 DNA. The library is plated, and plaques are lifted onto nylon membranes. The membranes are hybridized with the insert from clone 53.1 (1.9 kb) under normal stringency conditions. Six hybridizing plaques are chosen for further analysis.

EXAMPLE 10

DEMONSTRATION OF TELOMERASE ACTIVITY USING HT-1 AND TELOMERASE VARIANTS

- Full-length hT-1 sequence is cloned into an expression vector and the resulting protein is assayed for telomerase activity. Vector pRc/CMV2 (Invitrogen, Carlsbad, CA) is a eukaryotic expression vector that has a multi-cloning site positioned between a promoter, the RSV LTR, and a polyadenylation signal and transcription termination sequences from the bovine growth hormone gene. Telomerase sequence in which Leu49 codon was converted to a Met codon was inserted into pRc/CMV2. One clone, pHTC51, is chosen for further study. The DNA sequence of the 5' junction was determined and confirmed the orientation of the insert. Subsequently, the sequence of the 3' junction was determined and showed a deletion of the polyA signal, but no deletion of telomerase coding sequence.

- The clone is transfected into HeLa GM847 cells at passages 44 and 68, SUSM-1 cells at passage 18, and RKF-T/A6 cells at passage 40. Cell extracts are

assayed for telomerase activity by the TRAP assay as described herein. As shown in Figure 12, a ladder of products indicative of telomerase activity is seen at the 1:100 dilution of extract from SUSM-1 cells and is not seen in control cells. A ladder is not readily detectable at the higher concentration of extract, which may be due to nuclease activity in the extract.

Three telomerase variants are constructed: pAKI.4 is telomerase with the beta region spliced out (Figure 13); pAKI.7 is telomerase with the alternative C-terminus insert 3 (Figure 14); and pAKI.14 is telomerase with the alpha region spliced out (Figure 15). The 5' end of the telomerase gene was inserted into each of these three vectors and the inserts moved to pCIneo expression vector. The variants, along with reference telomerase in pCIneo are transiently transfected into GM847 cells, which are ALT cells having no detectable telomerase activity but which express the RNA subunit. Cell extracts are tested in a TRAP assay. The reference telomerase exhibits activity, as well as the telomerase with insert 3 (pAKI.7 insert), but the other variants do not express activity.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

We claim:

1. An isolated nucleic acid molecule encoding vertebrate telomerase.
2. The isolated nucleic acid molecule according to claim 1 wherein said vertebrate is a human.
3. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule comprises the sequence presented in Figure 1, or hybridizes under normal stringency conditions to the complement of the sequence presented in Figure 1, provided that the nucleic acid molecule is not EST AA281296.
4. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule encodes the amino acid sequence presented in Figure 1 or 11, or variant thereof.
5. An isolated nucleic acid molecule encoding any of the amino acid sequences presented in Figure 11, or hybridizes under normal stringency conditions to the complement of the sequences thereof, provided that the nucleic acid molecule is not EST AA281296.
6. An isolated nucleic acid molecule comprising any of the sequences presented in Figure 10, or hybridizes under normal stringency conditions to the complement of the sequences thereof.
7. An oligonucleotide comprising from 10 to 100 contiguous nucleotides from the sequence presented in Figure 1 or its complement.

8. An oligonucleotide comprising from 10 to 100 contiguous nucleotides from the sequences presented in Figure 10 or the complements thereof.

9. The oligonucleotide of either of claims 7 or 8, wherein the oligonucleotide is labeled.

10. The oligonucleotide of claim 9, wherein the label is a radiolabel, a chemiluminescent label, or biotin.

11. An expression vector, comprising a heterologous promoter operably linked to a nucleic acid molecule according any of claims 1-6.

12. The expression vector of claim 11, wherein the vector is selected from the group consisting of bacterial vectors, retroviral vectors, adenoviral vectors and yeast vectors.

13. A host cell containing a vector according to either claims 11 or 12.

14. The host cell of claim 13, wherein the cell is selected from the group consisting of human cell, monkey cell, mouse cell, rat cell, yeast cell and bacterial cell.

15. The host cell of claim 13, wherein the cell is a human cell.

16. An isolated protein comprising a vertebrate telomerase protein.

17. The protein of claim 16, wherein the vertebrate is a human.

18. The protein of claim 16, wherein the protein comprises the amino acid sequence presented in Figure 1 or 11, or variant thereof.

19. A portion of a vertebrate telomerase protein.
20. The portion of claim 19, wherein the amino acid sequence of the portion is presented in Figure 1.
21. The portion of claim 19, wherein the amino acid sequence of the portion is presented in Figure 11.
22. The portion of claim 19, wherein the portion is from 10 to 100 amino acids long.
23. An antibody that specifically binds to the protein according to either claim 16 or 19.
24. An antibody that specifically binds to a polypeptide encoded by a sequence selected from the group consisting of region 1, region α , region β , region 2 and region 3.
25. The antibody according to claim 24, wherein the antibody is a monoclonal antibody.
26. -A hybridoma that produces an antibody according to claim 14.
27. A nucleic acid probe that is capable of specifically hybridizing to a nucleic acid molecule encoding a vertebrate telomerase under conditions of normal stringency, provided that the probe does not hybridize to nucleotides 1624-2012 presented in Figure 1.
28. The probe of claim 27, wherein the probe is from 12 to 200 nucleotides long.

29. The probe of claim 27, wherein the probe is from 20 to 50 nucleotides long.

30. The probe of claim 17, wherein the nucleic acid molecule has the sequence presented in Figure 1 or its complement thereof.

31. The probe of claim 17, wherein the nucleic acid molecule is labeled.

32. A pair of oligonucleotide primers capable of specifically amplifying all or a portion of a nucleic acid molecule encoding human telomerase.

33. The primers of claim 32, wherein the nucleic acid molecule comprises the sequence presented in Figure 1 or its complement.

34. The primers of claim 32, wherein the nucleic acid molecule comprises any of the sequences presented in Figure 11 or the complements thereof.

35. The primers of claim 32, wherein the pair of primers is capable of specifically amplifying sequence comprising all or a part of region 1, region α , region β , region 2, region 3 region X or region Y.

36. The primers of claim 35, wherein the primers flank nucleotide 222, 1950, 2131-2166, 2287-2468, 2843, or 3157 as presented in Figure 1.

37. The primers of claim 36, wherein only one of each primer pair flanks nucleotide 222, 1950, 2131-2166, 2287-2468, 2843, or 3157 as presented in Figure 1 and the other primer of the pair has sequence corresponding to one of the sequences presented in Figure 10 or complements thereof.

38. A pair of oligoprimers capable of specifically amplifying genomic sequence presented in Figure 10, wherein the primers amplify more than nucleotides 1 to 38.

39. An oligonucleotide that hybridizes specifically to a nucleic acid sequence in region 1, region α , region β , region 2, region 3 region X or region Y.

40. The oligonucleotide of claim 39, wherein the oligonucleotide is from 15 to 36 bases.

41. A method of diagnosing cancer in a patient, comprising preparing tumor cDNA and amplifying the tumor cDNA using primers that specifically amplify human telomerase nucleic acid sequence, wherein the detection of telomerase nucleic acid sequences is indicative of a diagnosis of cancer.

42. The method of claim 41, further comprising comparing the amount of amplified telomerase sequence to a control, wherein increase telomerase nucleic acid sequences over the control is indicative of a diagnosis of cancer.

43. The method of claim 41, wherein the primers span region 1, region α , region β , region 2, region 3 region X or region Y, wherein the pattern of amplification is indicative of a diagnoses of cancer.

44. The method of claim 43, wherein the primers are Htel Intron T and Htel 723B.

45. The method of claim 44, wherein the primers are Htel335T and Htel1022B.

46. A method of determining a pattern of telomerase RNA expression in cells, comprising preparing cDNA from mRNA isolated from the cells, amplifying the cDNA

using primers according to claim 35, therefrom determining the pattern of telomerase RNA expression.

47. The method of claim 46, further comprising detecting the amplified product by hybridization with an oligonucleotide having all or part of the sequence of region 1, region α , region β , region 2, region 3 region X or region Y.

48. A method of diagnosing cancer in a patient, comprising determining a pattern of telomerase RNA expression, comprising amplifying telomerase from cDNA synthesized from tumor RNA, and detecting the amplified product by hybridization with an oligonucleotide having all or part of the sequence of region 1, region α , region β , region 2, region 3 region X or region Y, therefrom determining the pattern of telomerase RNA expression, wherein the pattern is indicative of a diagnosis of cancer.

49. The method of claim 48, further comprising comparing the pattern to a pattern obtained from a reference cancer.

50. A non-human transgenic animal whose cells contain a vertebrate telomerase gene that is operably linked to a promoter effective for the expression of the gene.

51. The animal of claim 50, wherein the animal is a mouse.

52. The animal of claim 50, wherein the promoter is tissue-specific.

53. The animal of claim 50, wherein the telomerase gene is any of the nucleic acid sequences presented in Figure 11.

54. A mouse, whose cells have an endogenous telomerase gene disrupted by homologous recombination with a nonfunctional telomerase gene, wherein the mouse is unable to express endogenous telomerase.

55. An inhibitor of vertebrate telomerase activity, wherein the inhibitor binds to telomerase and is not a nucleoside analogue.

56. The inhibitor of claim 55, wherein the vertebrate is a human.

57. The inhibitor of claim 55, wherein the inhibitor is antisense nucleic acid complementary to human telomerase mRNA.

58. The inhibitor of claim 57, wherein the antisense is complementary to region α , region β , region 2, region 3 or region X.

59. The inhibitor of claim 55, wherein the inhibitor is a ribozyme.

60. A method of treating cancer, comprising administering to a patient a therapeutically effective amount of an inhibitor according to claim 55.

61. A nucleic acid molecule comprising the sequence selected from the set consisting of sequences selected from region 1, region α , region β , region 2 or region 3 as presented in Figure 10 and variants thereof.

62. A method of identifying an effector of telomerase activity comprising:

(a) adding a candidate effector to a mixture of telomerase protein, RNA component and template, wherein the telomerase protein is encoded by an isolated nucleic acid molecule according to claim 1;

(b) detecting telomerase activity; and

(c) comparing the amount of activity in step (b) to the amount of activity in a control mixture without candidate effector, therefrom identifying an effector.

63. The method of claim 62, wherein the effector is an inhibitor.

64. the method of claim 62, wherein the nucleic acid molecule encodes human telomerase.

(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)
(n)
(o)
(p)
(q)
(r)
(s)
(t)
(u)
(v)
(w)
(x)
(y)
(z)

VERTEBRATE TELOMERASE GENES AND PROTEINS
AND USES THEREOF

ABSTRACT OF THE DISCLOSURE

Nucleic acid molecules encoding vertebrate telomerase are provided. Gene products, expression vectors and host cells suitable for expressing telomerase are also provided. Methods for identifying inhibitors of telomerase activity and inhibitor compositions are disclosed.

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HUMAN TELOMERASE

ATGCCGCGCGCTCCCCGCTGCCGAGCCGTGCGCTCCCTGCTGCGCAGCCACTACCGCGAG	60
MetProArgAlaProArgCysArgAlaValArgSerLeuLeuArgSerHisTyrArgGlu	20
GTGCTGCCGCTGGCCACGTTCTGTCGGCGCCCTGGGGCCCCAGGGCTGGCGGCTGGTGACAG	120
ValLeuProLeuAlaThrPheValArgArgLeuGlyProGlnGlyTrpArgLeuValGln	40
CGCGGGGACCCGGCGGCTTTCGCGCGCTGGTGGCCAGTGCCTGGTGTGCGTGCCCTGG	180
ArgGlyAspProAlaAlaPheArgAlaLeuValAlaGlnCysLeuValCysValProTrp	60
GACGCACGGCGCCCCCCCCCGCGCCCCCTCCTTCCGCCAGGTGTCCTGCCTGAAGGAGCTG	240
AspAlaArgProProProAlaAlaProSerPheArgGlnValSerCysLeuLysGluLeu	80
GTGCCCGAGTGCTGCAGAGGCTGTGCGAGCGCGCGCGAAGAACGTGCTGGCCTTCGGC	300
ValAlaArgValLeuGlnArgLeuCysGluArgGlyAlaLysAsnValLeuAlaPheGly	100
TTGCGGCTGCTGGACGGGGCCCCCGGGGGCCCCCGAGGCTTTCACCACCAGCGTGCGC	360
PheAlaLeuLeuAspGlyAlaArgGlyGlyProProGluAlaPheThrThrSerValArg	120
AGCTACCTGCCCAACACGGTGACCGACGCACTGCGGGGAGCGGGGCGTGGGGGCTGCTG	420
SerTyrLeuProAsnThrValThrAspAlaLeuArgGlySerGlyAlaTrpGlyLeuLeu	140
TTGCGCGCGTGGGCGACGACGTGCTGTTTCACTGCTGGCAGCGTGCGCGCTCTTTGTG	480
LeuArgArgValGlyAspAspValLeuValHisLeuLeuAlaArgCysAlaLeuPheVal	160
CTGGTGGCTCCAGCTGCGCCTACCAGGTGTGCGGGCGCGCTGTACCAGCTCGGCGCT	540
LeuValAlaProSerCysAlaTyrGlnValCysGlyProProLeuTyrGlnLeuGlyAla	180
GCCACTCAGGCCCCGCCCCGCCACACGCTAGTGGACCCCGAAGGCGTCTGGGATGCGAA	600
AlaThrGlnAlaArgProProProHisAlaSerGlyProArgArgArgLeuGlyCysGlu	200
CGGGCTGGAACCATAGCGTCAGGGAGGCGGGGTCCCCCTGGGCTGCCAGCCCCGGGT	660
ArgAlaTrpAsnHisSerValArgGluAlaGlyValProLeuGlyLeuProAlaProGly	220
GCGAGGAGGCGGGGGCAGTGCCAGCCGAAGTCTGCCGTTGCCCAAGAGGCCAGGCGT	720
AlaArgArgArgGlyGlySerAlaSerArgSerLeuProLeuProLysArgProArgArg	240

FIG. 1A

GGCGCTGCCCTGAGCCGGAGCGGACGCCGTTGGGCAGGGGTCTCTGGGCCACCCGGGC	780
GlyAlaAlaProGluProGluArgThrProValGlyGlnGlySerTrpAlaHisProGly	260
AGGACGCGTGGACCGAGTGACCGTGGTTTCTGTGTGGTGTACCTGCCAGACCCGCCGAA	840
ArgThrArgGlyProSerAspArgGlyPheCysValValSerProAlaArgProAlaGlu	280
GAAGCCACCTCTTTGGAGGGTGCGCTCTCTGGCACGCGCACTCCCACCCATCCGTGGGC	900
GluAlaThrSerLeuGluGlyAlaLeuSerGlyThrArgHisSerHisProSerValGly	300
CGCCAGCACACGCGGGCCCCCATCCACATCGCGGCCACCACGTCCCTGGGACACGCCT	960
ArgGlnHisHisAlaGlyProProSerThrSerArgProProArgProTrpAspThrPro	320
TGTCCCCCGGTGTAGCCCGAGACCAAGCACTTCCTCTACTCCTCAGGCAGCAAGGAGCAG	1020
CysProProValTyrAlaGluThrLysHisPheLeuTyrSerSerGlyAspLysGluGln	340
CTGCGGCCCTCCTTCTACTCAGCTCTCTGAGGCCAGCCTGACTGGCGCTCGGAGGCTC	1080
LeuArgProSerPheLeuLeuSerSerLeuArgProSerLeuThrGlyAlaArgArgLeu	360
GTGGAGACCATCTTTCTGGGTTCAGGCCCTGGATGCCAGGGACTCCCCGCAGGTTGCC	1140
ValGluThrIlePheLeuGlySerArgProTrpMetProGlyThrProArgArgLeuPro	380
CGCCTGCCCCAGCGCTACTGGCAAATGCGGCCCTGTTTCTGGAGCTGCTTGGGAACCAC	1200
ArgLeuProGlnArgTyrTrpGlnMetArgProLeuPheLeuGluLeuLeuGlyAsnHis	400
GCGCAGTGCCCTACGGGGTGCTCCTCAAGACGCACTGCCCCGTGCGAGCTGCGGTCACC	1260
AlaGlnCysProTyrGlyValLeuLeuLysThrHisCysProLeuArgAlaAlaValThr	420
CCAGCAGCCGGTGTCTGTGCCCGGAGAAGCCCCAGGGCTCTGTGGCGGCCCGCAGGAG	1320
ProAlaAlaGlyValCysAlaArgGluLysProGlnGlySerValAlaAlaProGluGlu	440
GAGGACACAGACCCCGTCGCTGGTGCAGCTGCTCCGCCAGCACAGCAGCCCTGGCAG	1380
GluAspThrAspProArgArgLeuValGlnLeuLeuArgGlnHisSerSerProTrpGln	460
GTGTACGGCTTCGTGCGGGCCTGCCTGCGCCGGCTGGTGCCCCAGGCCTCTGGGGCTCC	1440
ValTyrGlyPheValArgAlaCysLeuArgArgLeuValProProGlyLeuTrpGlySer	480
AGGCACAACGAACCGCGCTTCCTCAGGAACACCAAGAAGTTCATCTCCCTGGGAAGCAT	1500
ArgHisAsnGluArgArgPheLeuArgAsnThrLysLysPheIleSerLeuGlyLysHis	500

FIG. 1B

GCCAAGCTCTCGCTCAGGAGCTGACGTGGAAGATGAGCGTGC	1560
AlaLysLeuSerLeuGlnGluLeuThrTrpLysMetSerValArgAspCysAlaTrpLeu	520
CGCAGGAGCCCCAGGGGTGGCTGTGTCCGGCCGCAGAGCACCGTCTCGTGAGGAGATC	1620
ArgArgSerProGlyValGlyCysValProAlaAlaGluHisArgLeuArgGluGluIle	540
CTGGCCAAGTTCCTGCATGGCTGATGAGTGTGTACGTCGTCGAGCTGCTCAGGTCTTTC	1680
LeuAlaLysPheLeuHisTrpLeuMetSerValTyrValValGluLeuLeuArgSerPhe	560
TTTTATGTCACGGAGACCACGTTTCAAAGAACAGGCTCTTTTTCTACCGGAAGAGTGTC	1740
PheTyrValThrGluThrThrPheGlnLysAsnArgLeuPhePheTyrArgLysSerVal	580
TGGAGCAAGTTGCAAGCATTGGAATCAGACAGCACTTGAAGAGGGTGACAGTCGGGGAG	1800
TrpSerLysLeuGlnSerIleGlyIleArgGlnHisLeuLysArgValGlnLeuArgGlu	600
CTGTCGGAAGCAGAGGTGAGCAGCATCGGGAAGCCAGGCCCGCCCTGCTGACGTCCAGA	1860
LeuSerGluAlaGluValArgGlnHisArgGluAlaArgProAlaLeuLeuThrSerArg	620
CTCCGCTTCATCCCCAAGCCTGACGGGCTCGGGCCGATTGTGAACATGGACTACGTCGTG	1920
LeuArgPheIleProLysProAspGlyLeuArgProIleValAsnMetAspTyrValVal	640
GGAGCCAGAACGTTCCGCAGAGAAAAGAGGGCCGAGCGTCTCACCTCGAGGGTGAAGGCA	1980
GlyAlaArgThrPheArgArgGluLysArgAlaGluArgLeuThrSerArgValLysAla	660
CTGTTACAGCGTGCTCAACTACGAGCGGGCGCGGCCCGCCCTCCTGGGCGCCTCTGTG	2040
LeuPheSerValLeuAsnTyrGluArgAlaArgArgProGlyLeuLeuGlyAlaSerVal	680
CTGGGCCTGGACGATATCCACAGGGCCTGGCGCACCTTCGTGCTGCGTGTGCGGGCCCAG	2100
LeuGlyLeuAspAspIleHisArgAlaTrpArgThrPheValLeuArgValArgAlaGln	700
GACCCGCCGCTGAGCTGTACTTTGTCAAGGTGGATGTGACGGCGCCTACGACACCATC	2160
AspProProProGluLeuTyrPheValLysValAspValThrGlyAlaTyrAspThrIle	720
CCCCAGGACAGGCTCACGGAGGTTCATCGCCAGCATCATCAAACCCAGAACACGTACTGC	2220
ProGlnAspArgLeuThrGluValIleAlaSerIleIleLysProGlnAsnThrTyrCys	740
GTGCGTCGGTATGCCGTGGTCCAGAAGGCCGCCCATGGGCACGTCCGCAAGGCCTTCAAG	2280
ValArgArgTyrAlaValValGlnLysAlaAlaHisGlyHisValArgLysAlaPheLys	760

FIG. 1C

AGCCACGTCCTCTACCTTGACAGACCTCCAGCCGTACATGCGACAGTTCGTGGCTCACCTG SerHisValSerThrLeuThrAspLeuGlnProTyrMetArgGlnPheValAlaHisLeu	2340 780
CAGGAGACCAGCCCCGCTGAGGGATGCCGTCGTCATCGAGCAGAGCTCCTCCCTGAATGAG GlnGluThrSerProLeuArgAspAlaValValIleGluGlnSerSerSerLeuAsnGlu	2400 800
GCCAGCAGTGGCCTCTTTCGACGTCTTCTACGCTTCATGTGCCACCACGCCGTGCGCATC AlaSerSerGlyLeuPheAspValPheLeuArgPheMetCysHisHisAlaValArgIle	2460 820
AGGGGCAAGTCTACGTCCAGTCCAGGGGATCCCGCAGGGCTCCATCCTCTCCACGCTG ArgGlyLysSerTyrValGlnCysGlnGlyIleProGlnGlySerIleLeuSerThrLeu	2520 840
CTCTGCAGCCTGTGTCTACGGCGACATGGAGAACAAGCTGTTTGCGGGGATTTCGGCGGGAC LeuCysSerLeuCysTyrGlyAspMetGluAsnLysLeuPheAlaGlyIleArgArgAsp	2580 860
GGGCTGCTCCTGCGCTTGGTGGATGATTCTTGTGGTGACACCTCACCACGCGG GlyLeuLeuLeuArgLeuValAspAspPheLeuLeuValThrProHisLeuThrHisAla	2640 880
AAAACCTTCTCAGGACCTGGTCCGAGGTGTCCCTGAGTATGGCTGCGTGGTGAAGTCTG LysThrPheLeuArgThrLeuValArgGlyValProGluTyrGlyCysValValAsnLeu	2700 900
CGGAAGACAGTGGTGAAGTTCCTGTAGAAGACGAGGCCCTGGGTGGCACGGCTTTTGGTT ArgLysThrValValAsnPheProValGluAspGluAlaLeuGlyGlyThrAlaPheVal	2760 920
CAGATGCCGGCCACGGCCTATTTCCCTGGTGGCGCCTGCTGTGGATACCCGGACCCCTG GlnMetProAlaHisGlyLeuPheProTrpCysGlyLeuLeuLeuAspThrArgThrLeu	2820 940
GAGGTGCAGAGCGACTACTCCAGCTATGCCCGGACCTCCATCAGAGCCAGTCTCACCTTC GluValGlnSerAspTyrSerSerTyrAlaArgThrSerIleArgAlaSerLeuThrPhe	2880 960
AACCGCGGCTTCAAGGCTGGGAGGAACATGCGTCGCAAACTCTTTGGGGTCTTGCGGGCTG AsnArgGlyPheLeuAlaGlyArgAsnMetArgArgLysLeuPheGlyValLeuArgLeu	2940 980
AAGTGTACAGCCTGTTTCTGGATTGTCAGGTGAACAGCCTCCAGACGGTGTGCACCAAC LysCysHisSerLeuPheLeuAspLeuGlnValAsnSerLeuGlnThrValCysThrAsn	3000 1000
ATCTACAAGATCCTCCTGCTGCAGGCGTACAGGTTTCACGCATGTGTGCTGCAGCTCCCA IleTyrLysIleLeuLeuLeuGlnAlaTyrArgPheHisAlaCysValLeuGlnLeuPro	3060 1020

FIG. 1D

TTTCATCAGCAAGTTTGAAGAACCCACATTTTTCCTGCGCGTCATCTCTGACACGGCC	3120
PheHisGlnGlnValTrpLysAsnProThrPhePheLeuArgValIleSerAspThrAls	1040
TCCCTCTGCTACTCCATCCTGAAAGCCAAGAACGCAGGGATGTCGCTGGGGGCCAAGGGC	3180
SerLeuCysTyrSerIleLeuLysAlaLysAsnAlaGlyMetSerLeuGlyAlaLysGly	1060
GCCGCCGGCCCTCTGCCCTCCGAGGCCGTGCAGTGGCTGTGCCACCAAGCATTCCTGCTC	3240
AlaAlaGlyProLeuProSerGluAlaValGlnTrpLeuCysHisGlnAlaPheLeuLeu	1080
AAGCTGACTCGACACCGTGTCACTACGTGCCACTCCTGGGGTCACTCAGGACAGCCCAG	3300
LysLeuThrArgHisArgValThrTyrValProLeuLeuGlySerLeuArgThrAlaGln	1100
ACGCAGCTGAGTCGGAAGCTCCCGGGGACGACGCTGACTGCCCTGGAGGCCGACGCCAAC	3360
ThrGlnLeuSerArgLysLeuProGlyThrThrLeuThrAlaLeuGluAlaAlaAlaAsn	1120
CCGGCACTGCCCTCAGACTTCAAGACCATCCTGGACTgatggccaccgcccacagccag	3420
ProAlaLeuProSerAspPheLysThrIleLeuAsp	1132
Gccgagagcagacaccagcagccctgtcacgccgggctctacgtcccaggggagggagggg	3480
Cggccacacccaggcccgacccgtgggagtcctgaggcctgagtgagtggttggccgag	3540
gcctgcattgtccggctgaaggctgagtgccggctgaggcctgagcagtgctccagccaa	3600
gggctgagtgctccagcacacctgccgtcttcacttccccacaggctggcgctcggtcca	3660
ccccaggccagcttttccctcacaggagccggcttccactccccacataggaatagtc	3720
catccccagattcgccattgttcacccctcgccctgccctcctttgccctccaccccccac	3780
catccagggtggagaccctgagaaggaccctgggagctctgggaatttggagtgaccaaag	3840
gtgtgccctgtacacaggcgaggaccctgcacstggatgggggtccctgtgggtcaaatt	3900
ggggggaggtgctgtgggagtaaaatactgaatatatgagtttttcagttttgaaaaaaa	3960
aaaa	3964

FIG. 1E

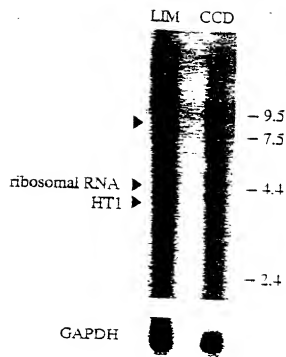


FIG. 3

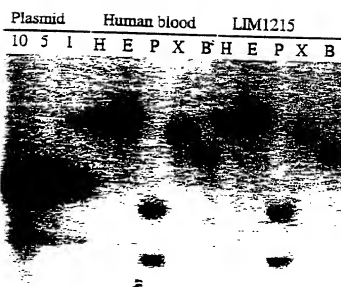


FIG. 4

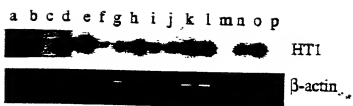


FIG. 5



FIG. 6

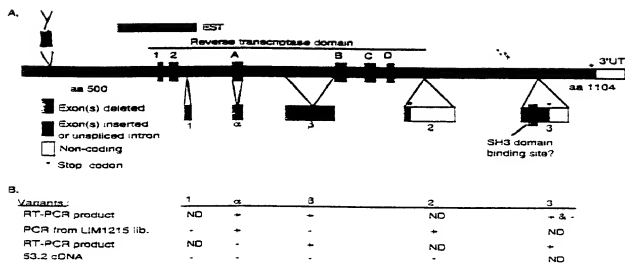


FIG. 7A and 7B

[illegible]

FIG. 7C



FIG. 8

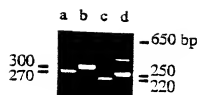


FIG. 9

sequence "i" 104-105 bases

GGCCTCCCCGGGGTCGGCGTCCGGCTGGGGTTGAGGGCGGGCCGGGGGAACCGAG
GlyLeuProGlyValGlyValArgLeuGlyLeuAlaAlaGlyGlyAsnGln
AlaSerProGlySerAlaSerGlyTrpGly * GlyArgProGlyGlyThrSer
ProProArgGlyArgProAlaGlyValGluGlyGlyArgGlyGluProAla

CGACATCGCGAGAGCAGCGCAGGCGACTCAGGGCGCTTCCCCCGCAGGTG
ArgHisAlaGluSerSerAlaGlyAspSerGlyArgPheProArgArg
AspMetArgArgAlaAlaGlnAlaThrGlnGlyAlaSerProAlaGly
ThrCysGlyGluGlnArgArgArgLeuArgAlaLeuProProGlnVal

sequence "l" 38 bases

GTGGCTGTGCTTTGGTTTAACTTCCTTTTAAACCAGAA
ValAlaValLeuTrpPheAsnPheLeuPheAsnGlnLys

sequence "α" 36 bases

GTGGATGTGACGGGCGCGTACGACACCATCCCCCAG
ValAspValThrGlyAlaTyrAspThrIleProGln

sequence "β" 182 bases

GTCTCTACCTTGACAGACCTCCAGCCGTACATGCGACAGTTCTGTGGCTCACCTG
ValSerThrLeuThrAspLeuGlnProTyrMetArgGlnPheValAlaHisLeu

CAGGAGACCAAGCCCCGCTGAGGGATGCCGTCGTCATCGAGCAGAGCTCCTCCCTG
GlnGluThrSerProLeuArgAspAlaValValIleGluGlnSerSerSerLeu

AATGAGGCCAGCAGTGGCCTCTTCGACGTCTTCCTACGCTTCATGTGCCACCAC
AsnGluAlaSerSerGlyLeuPheAspValPheLeuArgPheMetCysHisHis

GCCGTGCGCATCAGGGGCAA
AlaValArgIleArgGlyLys

partial sequence "2" unknown length

GTGAGCGCACCTGGCCGGAAGTGGAGCCTGTGCCCGGCTGGGGCAGGTGCTGCTGCAG
Ter

GGCCGTTGCGTCCACCTCTGCTTCCGTGTGGGGCAGGCGACTGCCAATCCCAAAGGGT
CAGATGCCACAGGGTGCCCTCGTCCCATCTGGGGCTGAGCACAAATGCATCTTCTG
TGGGAGTGAGGGTGCCTCACAACGGGAGCAGTTTCTGTGCTATTTTGGTAA..

sequence "3" 159 bases

CCGAAGAAAAACATTTCTGTCTGACTCCTGCGGTGCTTGGGTGGGACAGCCAGAG
AlaGluGluAsnIleSerValValThrProAlaValLeuGlySerGlyGlnProGlu

ATGGAGCCACCCCGCAGACCGTCGGGTGTGGGCAGCTTTCGGGTGTCTCCTGGGAGG
MetGluProProArgArgSerGlyValGlySerPheProValSerProGlyArg

GGAGTTGGGCTGGGCTGTGACTCCTCAGCCCTCTGTTTTCCCCCAG
GlyValGlyLeuGlyLeu *

FIG. 10A

sequence "X" unknown length

...GACAGTCACCAGGGGGGTTGACCGCCGGACTGGGCGTCCCCAGGGTTGACTATAGGA
CCAGGTGTCCAGGTGCCCTGCAAGTAGAGGGGCTCTCAGAGGCGTCTGGCTGGCATGG
GTGGACGTGGCCCCGGGCATGGCCTTCTGCGTGTGCTGCCGTGGGTGCCCTGAGCCCT
CACTGAGTCGGTGGGGGCTTGTGGCTTCCCGTGAGCTTCCCCCTAGTCTGTTGTCTGG
CTGAGCAAGCCTCCTGAGGGGCTCTCTATTG

partial sequence of genomic intron (approximately 2.7 kb)

GTGGCTGTGCTTTGGTTTAACTTCCTTTTTTAACCAAGTGCCTTTGAGCCCCACATT
TGGTATCAGCTTAGATGAAGGGCCCCGGAGGAGGGGCCACGGGACACAGCCAGGGCCAT
GGCACGGCGCCCACCCATTGTGCGCACAGTGAGGTGGCCGAGGTGCCGGTGCCCTCCA
GAAAGCAGCGTGGGGGTGTAGGGGGAGCTCCTGGGGCAGGGAC....

FIG. 10B

[illegible]

4

FIG. 11A

[illegible]

FIG. 11B

[illegible][illegible]

GTCCTACGTCCAGTG
 V L R P V

CAGGGGATCCCGCAGGCGTCGCATCTCTGTCCAGCTGCTCTGCGAGCTGTGCTACGGGACATGGAGAACAGCTCTTTCGGGGATTCCGGGGACGGGCTGCTCTGCTCTTGTGGA
 P G D P A G L H P L H A A L Q P V L R R H G S Q A V C G D S A G R A A P A P V G
 TGATTCTGTGTGGTGACACTCACCTCACCCAGCGGAAAGCTTCTCAGGACCTGCTCCAGCTGTCTCCCTAGTATGCTGCTGCTGTGAACCTCCGGAAGACAGTGTGAACTTCC

Reference protein

ATGCCGCGCGCTCCCGCTGCCGAGCCGTGCGCTCCCTGCTGCGCAGCCACTACCGGAG	60
MetProArgAlaProArgCysArgAlaValArgSerLeuLeuArgSerHisTyrArgGlu	20
GTGCTCCCGCTGGCCACGTTCTGCGGCGCCTGGGGCCCCAGGGCTGGCGCTGGTGCAG	120
ValLeuProLeuAlaThrPheValArgArgLeuGlyProGlnGlyTrpArgLeuValGln	40
CGCGGGACCCCGCGCTTTCCGCGCGCTGGTGGCCAGTGCCTGGTGTGCTGCCCTGG	180
ArgGlyAspProAlaAlaPheArgAlaLeuValAlaGlnCysLeuValCysValProTrp	60
GACGCACGCGCGCCCCCGCGCCCTCCTTCGCGCAGGTGCTGCCTGAAGGAGCTG	240
AspAlaArgProProProAlaAlaProSerPheArgGlnValSerCysLeuLysGluLeu	80
GTGGCCCGAGTGTGCAGAGGCTGTGCGAGCGCGCGCGAAGAACGTGCTGGCCTTCGCG	300
ValAlaArgValLeuGlnArgLeuCysGluArgGlyAlaLysAsnValLeuAlaPheGly	100
TTCCGCTCTGGACGCGGCGCGCGGCGCGCGCGAGGCCTTCAACACAGCGTGGCGC	360
PheAlaLeuLeuAspGlyAlaArgGlyGlyProProGluAlaPheThrThrSerValArg	120
AGCTACTGCGCAACACAGGTGACGACGCACTGCGGGGAGCGGGGCTGGGGCTGCTG	420
SerTyrLeuProAsnThrValThrAspAlaLeuArgGlySerGlyAlaTrpGlyLeuLeu	140
TTGCGCGCGCTGGGACGACGCTGCTGCTTCCCTGCTGCGCAGCTGCGCGCTCTTTGTG	480
LeuArgArgValGlyAspValLeuValHisLeuLeuAlaArgCysAlaLeuPheVal	160
CTGGTGGCTCCCAGCTGCGCCTACCAGGTGTGCGGGCGCGCGCTGTACAGCTCGCGCT	540
LeuValAlaProSerCysAlaTyrGlnValCysGlyProProLeuTyrGlnLeuGlyAla	180
GCCACTCAGGCGCGGCGCGCGCAACGCTAGTGACCCGAAGCGCTCTGGGATGCGAA	600
AlaArgArgArgGlyProProProHisAlaSerGlyProArgArgArgLeuGlyCysGlu	200
CGGCGCTGGAACCATAGCGCTCAGGAGGCGGGGTCCCGCTGGCGCTGCCAGCCCGGGT	660
ArgAlaTrpAsnHisSerValArgGluAlaGlyValProLeuGlyLeuProAlaProGly	220
GCGAGGAGCGCGCGCGCGAGTGCCAGCCGAAGTCTGCGGTGCCCCAAGAGGCGCCAGGCT	720
AlaArgArgArgGlyGlySerAlaSerArgSerLeuProLeuProLysArgProArgArg	240
GGCGCTGCCCTGAGCCGAGCGGAGCGCCCGTTGGGCGGGGTCTGGGCCACCCCGGCGC	780
GlyAlaAlaProGluProGluArgThrProValGlyGlnGlySerTrpAlaHisProGly	260
AGGACGCGTGACCGAGTGACCGTGGTTTCTGTGTGTGTACCTGCCAGACCGCGCGAA	840
ArgThrArgGlyProSerAspArgGlyPheCysValValSerProAlaArgProAlaGlu	280
GAAGCCACTCTTTGGAGGGTGCCTCTCTGGCACGCGCCACTCCACCCATCCGTGGGC	900
CysProProValLeuGlyAlaLeuSerGlyThrArgHisSerHisProSerValGly	300
CGCCAGCACGCGGGCGCGCCCATCCACATCGCGGCCACCACTCCCTGGGACACGCT	960
ArgGlnHisHisAlaGlyProProSerThrSerArgProProArgProTrpAspThrPro	320
TGTCGCCCGGTGTACGCGAGACCAAGCACTTCTCTACTCTCAGGCGACAAGGAGGAG	1020
CysProProValLeuAlaGluThrLysHisPheLeuTyrSerSerGlyAspLysGluGln	340
CTGCGGCGCTCTTCTACTCAGCTCTCTGAGGCGGAGCCTGACTGGCGCTCGGAGGCTC	1080
LeuArgProSerPheLeuLeuSerSerLeuArgProSerLeuThrGlyAlaArgArgLeu	360
GTGGAGACCATCTTTCTGGGTTCAGGCGCTGGATGCCAGGGACTCCCGCAGGTGGCCC	1140
ValGluThrIlePheLeuGlySerArgProTrpMetProGlyThrProArgArgLeuPro	380
CGCTGCCCGCAGCGCTACTGGCAATGCGGCGCTGTTCTGGAGCTGCTTGGGAACAC	1200
ArgLeuProGlnArgTyrTrpGlnMetArgProLeuPheLeuGluLeuLeuGlyAsnHis	400
GCGAGTGCGCTACGCGGTGCTCTCAAGACGCACTGCCCGCTGCGAGCTGCGGTCAAC	1260
AlaGlnCysProTyrGlyValLeuLeuLysThrHisCysProLeuArgAlaAlaValThr	420

FIG. 11D

CCAGCAGCCGGTGTCTGTGCCCGGGAGAAGCCCCAGGGCTCTGTGGCGGCCCCGAGGAG	1320
ProAlaAlaGlyValCysAlaArgGluLysProGlnGlySerValAlaAlaProGluGlu	440
GAGGACACAGACCCCCGTCGCTGTGCAGCTGTCTCCGCCAGCAGCAGCCCTGGCAG	1380
GluAspThrAspProArgArgLeuValGlnLeuLeuArgGlnHisSerSerProTrpGln	460
GTGTACGGCTTCGTGCGGGCCTGCCTGCGCGCGGTGGTCCCCCAGGCCTCTGGGGCTCC	1440
ValTyrGlyPheValArgAlaCysLeuArgArgLeuValProProGlyLeuTrpGlySer	480
AGGCACAACGAAACCGCTTCCTCAGGAACACCAAGAAGTTCATCTCCCTGGGAAGCAT	1500
ArgHisAsnGluArgArgPheLeuArgAsnThrLysLysPheIleSerLeuGlyLysHis	500
GCCAAAGCTCTCGCTGCAGGAGCTGACGTGGAAGATGAGCGTGCGGGCTCGCCTTGGCTG	1560
AlaLysLeuSerLeuGlnGluLeuThrTrpLysMetSerValArgAspCysAlaTrpLeu	520
CGCAGGAGCCAGGGGTTGGCTGTGTTCCGCGCCGAGAGCACCGCTCGCTGAGGAGATC	1620
ArgArgSerProGlyValGlyCysValProAlaAlaGluHisArgLeuArgGluGluIle	540
CTGGCCAACTTCCTGCACTGGCTGATGAGTGTGTACGTGCTGAGCTGCTCAGGTCTTTC	1680
LeuAlaLysLeuGlnHisTrpLeuMetSerValTyrValValGluLeuLeuArgSerPhe	560
TTTTATGTACGGAGACCACGTTTCAAAGAACAGGCTCTTTTTCTACCGGAAGAGTTC	1740
PheTyrValThrGluThrThrPheGlnLysAsnArgLeuPhePheTyrArgLysSerVal	580
TGGAGCAAGTTGCAAAGCATTGGAATCAGACAGCACTTGAAGAGGGTCAGCTCGCGGAG	1800
TrpSerLysLeuGlnSerIleGlyIleArgGlnHisLeuLysArgValGlnLeuArgGlu	600
CTGTGGAAGCAGAGGTGAGCGAGCATCGGGAAGCCAGSCCGCTGCTGACGTCCAGA	1860
LeuSerGluAlaGluValArgGlnHisArgGluAlaArgProAlaLeuLeuThrSerArg	620
CTCCGCTTCATCCCCAAGCCTGACGGGCTCGCGCGATTGTGAACATGGAATACGTCGTG	1920
LeuArgPheIleProLysProAspGlyLeuArgProIleValAsnMetAspTyrValVal	640
GGAGCCGAGACGTTCCGCAGAGAAAGAGGSCGAGCGTCTCACCTCGAGGGTGAAGGCA	1980
GlyAlaArgThrPheArgArgGluLysArgAlaGluArgLeuThrSerArgValLysAla	660
CTGTTACAGCTGTCTCAACTACGAGCGGGCGCGCGCCCGCGCTCTCTGGCGCTCTGTG	2040
LeuPheSerValLeuAsnTyrGluArgAlaArgArgProGlyLeuLeuGlyAlaSerVal	680
CTGGCGCTGGACGATATCCACAGGGCTGGCGCACCTTCGTGCTGCGTGTGCGGCGCCAG	2100
LeuGlyLeuAspAspIleHisArgAlaTrpArgThrPheValLeuArgValArgAlaGln	700
GACCCGCCCGCTGAGCTGTACTTTGTCAAGGTGGATGTGACGGGCGCTACGACACCATC	2160
AspProProProGluLeuTyrPheValLysValAspValThrGlyAlaTyrAspThrIle	720
CCCCAGGACAGGCTCACCGAGGTTCATCGCCAGCATCATCAAACCCAGAACACGTACTGC	2220
ProGlnAspArgLeuThrGluValIleAlaSerIleIleLysProGlnAsnThrTyrCys	740
GTGCGTCGCTATGCGGTGGTCCAGAGGCGCGCCATGGGCACGTCCGCAAGGCGCTCAAG	2280
ValArgArgTyrAlaValValGlnLysAlaAlaHisGlyHisValArgLysAlaPheLys	760
AGCCACGCTCTACCTTGACAGACCTCCAGCCGTACATGCGACAGTTCGTGGCTCACCTG	2340
SerHisValSerThrLeuThrAspLeuGlnProTyrMetArgGlnPheValAlaHisLeu	780
CAGGAGACACGCCGCTGAGGATGCGGTGTCATCGAGCAGAGCTCTCCCTGAATGAG	2400
GlnGluThrSerProLeuArgAspAlaValValIleGluGlnSerSerSerLeuAsnGlu	800
GCCAGCAGTGGCCTCTTCGACGTCTTCTACGCTTCATGTGCCACCACGCGTGGCATC	2460
AlaSerSerGlyLeuPheAspValPheLeuArgPheMetCysHisHisAlaValArgIle	820
AGGGGCAAGTCTCTACGTCCAGTCCAGGGGATCCCGCAGGGCTCCATCTCTCCACGCTG	2520
ArgGlyLysSerTyrValGlnCysGlnGlyIleProGlnGlySerIleLeuSerThrLeu	840
CTCTGACGCTGTGCTACGGCGACATGGAGAACAAGCTGTTTTCGGGGATTCTGGCGGGAC	2580
LeuCysSerLeuCysTyrGlyAspMetGluAsnLysLeuPheAlaGlyIleArgArgAsp	860
GGGCTGCTCCTGCGTTTGGTGGATGATTCTTGTGTGTGACACCTCACCTCACCAAGCGG	2640

FIG. 11E

[illegible]

CCGAGAAAACATTCTGTCTGTGACTCCTGGGGTGCTGGGTC
E E N I L V V T P A V L G S

CCGAAGAAAAATTCTGCTGTGACTCTCGGGTCTTGGGTC
Z E N I L V V T P A V L G S
GGGACAGCCAGAGATGGAGCCACCCCGCAGACCGTGGGTGTGGGACGCTTCGGGTCTGGAGGGGAGTGGGCTGGGCTGTGACTCTCGAGCGCTCTGTCTTTTCCCCAG
G Q P S M E P P R R P S G V G S P P V S P G R G V G L G I . *

FIG. 11H

[illegible]

FIG. 11U

[illegible]

•

FIG. 11.J

[illegible]

CCGAAGAAACATTTCTGTCTGTAAGTCTTGGCGGTGCTTGGGTG
E E N I L V V T P A V L G S

FIG. 11K

[illegible]

4

FIG. 11L

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	

FIG. 11M

FIG. 11N

Reference protein (ver. 2)

ATGCCGCGCGCTCCCCGCTGCCGAGCCGTGGCTCCCTGCTGCGCAGCCACTACCGCGAG 60
 MetProArgAlaProArgCysArgAlaValArgSerLeuLeuArgSerHisTyrArgGlu 20
 GTGCTGCCGCTGGCCACGTTTCGTGCGGCGCCTGGGGCCCCAGGGCTGGCGGCTGGTGAG 120
 ValLeuProLeuAlaThrPheValArgArgLeuGlyProGlnGlyTrpArgLeuValGln 40
 CGCGGGGACCCGCGCGCTTTCCGCGCGCTGGTGGCCACTGCCTGGTGTGCGTGGCCTGG 180
 ArgGlyAspProAlaAlaPheArgAlaLeuValAlaGlnCysLeuValCysValProTrp 60
 GACGACGCGCGCCCCCGCGCGCCCCCTCTTCCGSCCAGGTG
 AspAlaArgProProProAlaAlaProSerPheArgGlnVal
 GCGCTCCCGCGGCTCGCGCTCGCGCTGGGTTGAGGCGCGCGCGGGGGAACAGCGACATGCGAGAGCAGCGCGAGCGCTCAGGCGCGCTCCCCCGCGAGGTG
 G L P F G V G R L G L R A A G G N Q R H A E S S A G D S G R F P R R
 A S P G S A S G W G * G R P G G T S D M R R A A Q A T Q G A S P A G
 P P R G R R P A G V E G G R G E P A T C G E Q R R R L R A L P P Q V
 TCCTGCGTGAAGGAGCTG 240
 SerCysLeuLysGluLeu 80
 GTGGCCGAGTGCTGCAGAGGCTGTGCGAGCGCGCGCGGAAGAACCTGCTGGCCTTCGGC 300
 ValAlaArgValLeuGlnArgLeuCysGluArgGlyAlaLysAsnValLeuAlaPheGly 100
 TTCGCGCTGCTGACGCGGGCCCGCGGGGGCCCCCGAGGCGCTTACCACAGCGTGGCGC 360
 PheAlaLeuLeuAspGlyAlaArgGlyGlyProProGluAlaPheThrThrSerValArg 120
 AGCTACCTGCCAACACCGTGACCGACGACTGCGGGGAGCGGGGCGTGGGGCTGTGCTG 420
 SerTyrLeuProAsnThrValThrAspAlaLeuArgGlySerGlyAlaTrpGlyLeuLeu 140
 TTGCGCGCGTGGGCGACGACGTGCTGGTTACCTGCTGGCACGCTGCGCGCTTTTGTG 480
 LeuArgAlaValGlyAspAspValLeuValHisLeuAlaAargCysAlaLeuPheVal 160
 CTGGTGGCTCCAGCTGCGGCTACCAGGTGTGCGGGCGCGCGCTTACCAGCTCGGCGCT 540
 LeuValAlaProSerCysAlaTyrGlnValCysGlyProProLeuTyrGlnLeuGlyAla 180
 GCCACTCAGCGCGCGCCCCCGCCACACGCTAGTGGACCCGAAGGCGCTTGGGATGCGAA 600
 AlaThrGlnAlaArgProProProHisAlaSerGlyProArgArgArgLeuGlyCysGlu 200
 CGGGCTCGGAACCATAGCGTCAGGGAGGCGGGGCTCCCCCTGGGCTGCGCAGCCCCGGT 660
 ArgAlaTrpAsnHisSerValArgGluAlaGlyValProLeuGlyLeuProAlaProGly 220
 CGCAGGAGGCGCGGGGCGAGTGCACCGGAAGTCTGCGGTTGCCAAGAGGCCAGCGGT 720
 ArgArgArgGlyGlySerAlaSerArgSerLeuProLysArgProArgArg 240
 GCGCGTCCGCTGAGCCGAGCGGAGCGCCGCTGGGCGAGGGTCTGGGCCACCCCGGGC 780
 GlyAlaAlaProGluProGluArgThrProValGlyGlnGlySerTrpAlaHisProGly 260
 AGGACGCGTGGACCGAGTGACCGTGGTTCTGTGTGTGTCACTGCGCAGACCGCCGAA 840
 ArgThrArgGlyProSerAspArgGlyPheCysValValSerProAlaArgProAlaGlu 280
 GAAGCCACCTCTTTGGAGGGTGGCGCTCTCTGGCACCGCGCACTCCACCCATCGTGGGC 900
 GluAlaProThrLeuGluGlyAlaLeuSerGlyThrArgHisSerHisProSerValGlu 300
 CGCCAGCACACCGCGGGCCCCCATCCACATCGCGGCCACACGTCCTGGGACACGCGCT 960
 ArgGlnHisHisAlaGlyProProSerThrSerArgProProArgProTrpAspThrPro 320
 TGTCCCGCGGTGACGCGAGACCAAGCACTTCTCTACTCTCTCAGGCGACAGGAGCAG 1020
 CysProValTyrAlaGluThrLysHisPheLeuTyrSerSerGlyAspLysGluGln 340
 CTGCGGCGCTCTCTCTACTCAGCTCTCTGAGGCCAGCCTGACTGGCGCTCGGAGGCTC 1080
 LeuArgProSerPheLeuLeuSerSerLeuArgProSerLeuThrGlyAlaArgArgLeu 360
 GTGGAGACCATCTTTCTGGGTTCCAGGCCCTGGATGCCAGGGACTCCCGCAGGTGGCCC 1140
 ValGluThrIlePheLeuGlySerArgProTrpMetProGlyThrProArgArgLeuPro 380
 CGCGTCCCCAGCGCTACTGGCAATGCGGCCCTGTGTTCTGGAGCTGCTTGGGAACCCAC 1200

FIG. 110

ArgLeuProGlnArgTyrTrpGlnMetArgProLeuPheLeuGluLeuLeuGlyAsnHis	400
GCGCAGTGCCTTACGGGGTCTCCTCAAGACGCACTGCCCGCTGCGAGCTGCGGTACCC	1260
AlaGlnCysProTyrGlyValLeuLeuLysThrHisCysProLeuArgAlaAlaValThr	420
CCAGCAGCGGTGTCTGTGCCCGGAGAAGCCCCAGGGCTCTGTGGCGCCCCCGAGGAG	1320
ProAlaAlaGlyValCysAlaArgGluLysProGlnGlySerValAlaAlaProGluGlu	440
GAGGACACAGACCCCGTCCGCTGGTGAGCTGCTCCGCGAGCACAGCACCCCTGGCAG	1380
GluAspThrAspProArgArgLeuValGlnLeuLeuArgGlnHisSerSerProTrpGln	460
GTGTACGGCTCTGTGCGGGCTGCTGCGCGGGCTGGTGCCCCAGGGCTCTGGGGCTCC	1440
ValTyrGlyPheValArgAlaCysLeuArgArgLeuValProProGlyLeuTrpGlySer	480
AGGCACAACGAACCGCGCTTCTCAGGAACACCAAGAAGTTCATCTCCCTGGGGAAGCAT	1500
ArgHisAsnGluArgArgPheLeuArgAsnThrLysLysPheIleSerLeuGlyLysHis	500
GCCAAGCTCTCGCTGCAAGAGCTGACGTGGAAGATGAGCGTGCGGGCTCGCTTGGCTG	1560
AlaLysLeuSerLeuGlnGluLeuThrTrpLysMetSerValArgAspCysAlaTrpLeu	520
CGCAGGAGCCCCAGGGTGGCTGTGTTCCGGCCGAGAGCACCGTCTGCTGAGGAGATC	1620
ArgArgSerProGlyValGlyCysValProAlaAlaGluHisArgLeuArgGluGluIle	540
CTGGCCAAGTTCCTGCACTGGCTGATGAGTGTGTACCTCGTCCAGCTGCTCAGGTCTTTC	1680
LeuAlaLysPheLeuHisTrpLeuMetSerValTyrValValGluLeuLeuArgSerPhe	560
TTTTATGTCAACGAGACACGTTTCAAAGAAGACGGCTCTTTTCTACCGGAAGAGTGC	1740
PheTyrValThrGluThrThrPheGlnLysAsnArgLeuPhePheTyrArgLysVal	580
TGGCAACAAGTTCGAAGCATTGGAATCAGACGCACTTGAAGAGGTGCACTGCGGGAG	1800
TrpSerLysLeuGlnSerIleGlyIleArgGlnHisLeuLysArgValGlnLeuArgGlu	600
CTGTCCGAAGCAGAGGTGAGGCAGCATCGGGAAGCCAGGCCCGCTCTGTCAGTTCAGA	1860
LeuSerGluAlaGluValArgGlnHisArgGluAlaArgProAlaLeuLeuThrSerArg	620
CTCCGCTTCATCCCCAAGCCTGACGGCTGCGGCCGATTGTGAACATGGACTACGTCTGT	1920
LeuArgPheIleProLysProAspGlyLeuArgProIleValAsnMetAspTyrValVal	640
GGAGCCAGAACGTTCCGCGAGAGAAAAGAGGCCGAGCGTCTCACCTCGAGGGTGAAGGCA	1980
GlyAlaArgThrPheArgArgGluLysArgAlaGluArgLeuThrSerArgValLysAla	660
CTGTTCAAGCTGCTCAACTACGAGCGGGCGGGCGCCCCGCGCTCTCTGGCGCCTCTGTG	2040
LeuPheSerValLeuAsnTyrGluArgAlaArgArgProGlyLeuLeuGlyAlaSerVal	680
CTGGGCTGGACGATATCCACAGGGCTGGCGCACCTCTGTGCTGCTGTGCGGGCCAG	2100
LeuGlyLeuAspAspIleHisArgAlaTrpArgThrPheValLeuArgValArgAlaGln	700
GACCCGCGCGCTGAGCTGTACTTTGTCAAGGTGGATGTGACGGCGCGTACGACACCATC	2160
ArgProProGluLeuTyrPheValLysValAspValThrGlyAlaTyrAspThrIle	720
CCCCAGACAGAGCTCAGGAGGTGCATCGCCAGCATCATCAACCCCGAAGACGTAAGTGC	2220
ProGlnAspArgLeuThrGluValIleAlaSerIleIleLysProGlnAsnThrTyrCys	740
GTGCGTCGTATGCGGTGCTCCAGAAGCGCGCCATGGGCACGTCCGCAAGCGCTTCAAG	2280
ValArgArgTyrAlaValValGlnLysAlaAlaHisGlyHisValArgLysAlaPheLys	760
AGCCACGCTCTTACCTTGACAGACCTCCAGCGGTACATGCGACAGTTCTGTGGCTCACCTG	2340
SerHisValSerThrLeuThrAspLeuGlnProTyrMetArgGlnPheValAlaHisLeu	780
CAGGAGACAGCGCGCTGAGGGATGCCGTGTCATCGAGCAGAGCTCTCCCTGAATGAG	2400
GlnGluThrSerProLeuArgAspAlaValValIleGluGlnSerSerSerLeuAsnGlu	800
GCCACAGTGGCGCTCTTCGACGTCTTCTACGCTTCATGTCCACCACGCGTGGCGCATC	2460
AlaSerSerGlyLeuPheAspValPheLeuArgPheMetCysHisHisAlaValArgIle	820
AGGGGCAAGTCTACGTCAGTGCCAGGGGATCCCGCAGGGCTCCATCTCTCCACGCTG	2520
ArgGlyLysSerTyrValGlnCysGlnGlyIleProGlnGlySerIleLeuSerThrLeu	840
CTCTCGAGCTGTGCTACGGCGCATGGAGAACAAGCTGTTTGGCGGGATTGCGGGGAC	2580

FIG. 11P

LeuCysSerLeuCysTyrGlyAspMetGluAsnLysLeuPheAlaGlyIleArgArgAsp	860
GGGCTGCTCCTGCGTTTGGTGGATGATTCTTGTTGGTGACACCTCACCTCACCCACGG	2640
GlyLeuLeuLeuArgLeuValAspAspPheLeuLeuValThrProHisLeuThrHisAla	880
AAACCTTCTCAGGACCTGGTCCGAGGTGTCCCTGAGTATGGCTGCGTGGTGAACCTG	2700
LysThrPheLeuArgThrLeuValArgGlyValProGluTyrGlyCysValValAsnLeu	900
CGAAGACAGTGGTGAACCTCCCTGTAGAAGACGAGGCCCTGGGTGGCACGGCTTTTGT	2760
ArgLysThrValValAsnPheProValGluAspGluAlaLeuGlyGlyThrAlaPheVal	920
CAGATGCCGCCACCGGCTATTCCCTGGTGGCGCTGCTGCTGGATACCGGACCCCTG	2820
GlnMetProAlaHisGlyLeuPheProTrpCysGlyLeuLeuAspThrArgThrLeu	940
GAGGTGCAGAGCGACTACTCCAGCTATGCCCGGACCTCCATCAGAGCCAGTCTCACCTTC	2880
GluValGlnSerAspTyrSerSerTyrAlaArgThrSerIleArgAlaSerLeuThrPhe	960
AACCGCGGCTTCAAGGCTGGGAGGAACATGCCCTCGCAAACCTCTTTGGGGTCTTCCGGCTG	2940
AsnArgGlyPheLysAlaGlyArgAsnMetArgArgLysLeuPheGlyValLeuArgLeu	980
AAGTGTACAGCCTGTTTCTGGATTTCAGGTGAACAGCCTCCAGACGGTGTGCACCAAC	3000
LysCysHisSerLeuPheLeuAspLeuGlnValAsnSerLeuGlnThrValCysThrAsn	1000
ATCTACAAGATCCTCCTGCTGCAGGCGTACAGTTTCACGCATGTGTGCTGCAGCTCCCA	3060
IleTyrLysIleLeuLeuLeuGlnAlaTyrArgPheHisAlaCysValLeuGlnLeuPro	1020
TTTCATCAGCAAGTTTGAAGAACCCACATTTTCTGCGCGTCATCTCTGACACGGCC	3120
PheHisGlnGlnValTrpLysAsnProThrPhePheLeuArgValIleSerAspThrAla	1040
TCCCTCTGCTACTCCATCCTGAAAGCCAAGAACGAGGATGTGCTGGGGGCCAAGGSC	3180
SerLeuCysTyrSerIleLeuLysAlaLysAsnAlaGlyMetSerLeuGlyAlaLysGly	1060
GCCGCCGCCCTCTGCCCTCCGAGGCCGTGCAGTGGCTGTGCCACCAAGCATTCTGCTC	3240
AlaAlaGlyProLeuProSerGluAlaValGlnTrpLeuCysHisGlnAlaPheLeuLeu	1080
AAGCTGACTGCACACCGTGTACCTACGTGCCACTCTGGGGTCACTCAGGACAGCCAG	3300
LysLeuThrArgHisArgValThrTyrValProLeuLeuGlySerLeuArgThrAlaGln	1100
ACGCAGCTGAGTCGGAAGCTCCCGGGGACGAGCTGACTGCCCTGGAGGCCGAGCCAAC	3360
ThrGlnLeuSerArgLysLeuProGlyThrThrLeuThrAlaLeuGluAlaAlaAlaAsn	1120
CCGCACTGCCCTCAGACTTCAAGACCATCCTGGAC	3420
ProAlaLeuProSerAspPheLysThrIleLeuAsp	1132

FIG. 11Q

[illegible]

GGAACCTGGGAGCTCTGGGAATTTGGAGTGACCAAGGTGTGCCCTTTACACAGGGGAGGACCTTGACCTGGATGGGGGTCCCTGTGGGTCAAAATTGGGGGGAGGTGCTGTGGGAGTAA
 AATACTGAAATATATGAGTTTTTCAGTTTTGA

10
 20
 30
 40
 50
 60
 70
 80
 90
 100
 110
 120
 130
 140
 150
 160
 170
 180
 190
 200

FIG. 11U

[illegible]

2

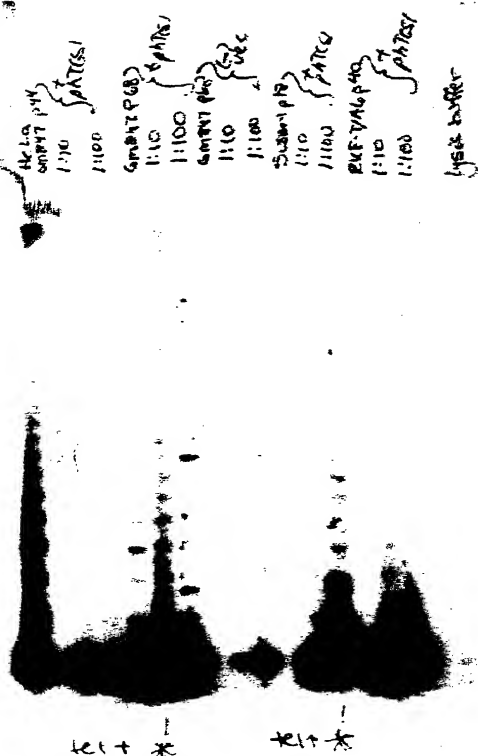


FIG. 12

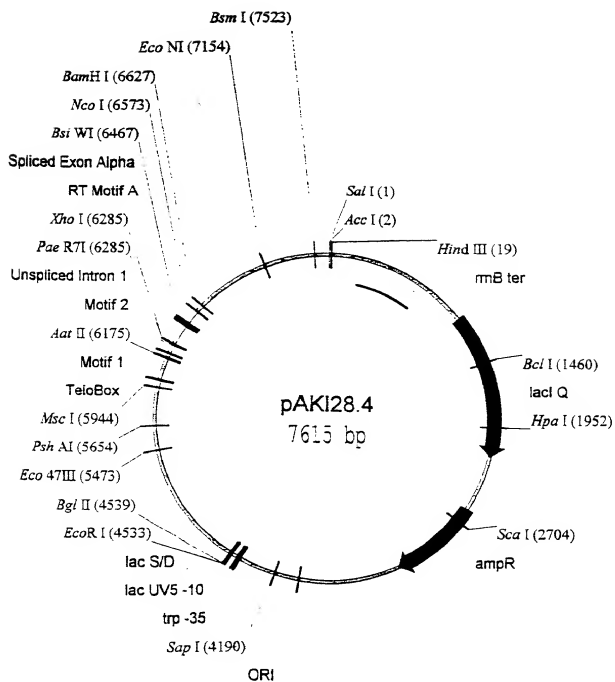


FIG. 13A

LOCUS pAKR28.4 7615 bp dsDNA Circular
 DEFINITION Human telomerase clone with exon beta spliced out

```

1  tcgacctgca ggcattgcaag cttggcactg gccgtcgttt tacaacgtcg tgactgggaa
61  aacctctggc ttacctcaact taatcgccct gcagcacate cccctttcgc cagctggcgt
121 aatagcgaa agggccgcac cgatcgccct tcccaacagt tgcgcagcct gaatggcgaa
181 tggcgccctga tgcggtatct tctcctttag catctgtgcg gtatttcaca ccgcataaat
241 tccccgtttt ggcggatgag agaagatctt cagcctgata cagattaaat cagaacgcag
301 aagcggctct ataaaaacaga atttgccctg cggcagtagc gcggtgggtcc cacctgaccc
361 catgccgaac tcagaagtga aacgcctgag cggcagtggt agtgtggggt ctccccatgc
421 gagagtggg aactgccagg catcaaatga aacgaaggcc tcagtgcgaa gactgggcct
481 ttcgttttat ctgttgtttg tcggtgaacg ctctcctgag taggacaaat ccgcggggag
541 cggattttga cgttgcaag caacggcccg gaggtgtggc ggcaggacgc ccgccataaa
601 ctgccaggca tcaaatgaag cagaaggcca tcctgacgga tggccttttt gcgtttctac
661 aaactcttcc tgtcgtcata tctacaagcc atccccccac agatagcgga aactagcttc
721 gtttttgcac caggaaagca gggaaattat ggtgcactct cagtacaaac tgctctgatg
781 ccgcatagtt aagccagccc cgacaccgcg caacaccgcg tgacgggctt

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FIG. 13B

841 gtctgctccc ggcattccgt tacagacaag ctgtgaccgt etccggggag tgcattgtgc
 901 agagggttttc accgtcatca ccgaaacgag cgagacgaaa gggccctcgt atacccttat
 961 ttttatagggt taatgtcatg ataataatgg ttctctagac gtgagggtct gtaccgcgaa
 1021 ccatcgatag gtgcataaac ttctcgcggt ttgcctgata gcgcccggaa gagagtgcaat
 1081 tcagggtgggt gaattgtgaaa ccagtaacgt tatccatggt cgcagagtat gcgggtgtct
 1141 cttattcagac ggtttcccgcg gtggtgaacc aggccagcca cgtttctcgc aaacccgggg
 1201 aaaaagtgga agcgggcgat gcgggagctga attacattcc caaccggcgt gcacaacaac
 1261 tggcgcgcaa acagtctgtg ctgattggcg ttgccacctc cagctctggc ctgcgcgcgc
 1321 cgtcgcaaat tgtcgcgcg attaaatctc gcggcgatca actgggtggc aggtgtgtgg
 1381 tgtcgatggt agaaccgaag gcgctcgaaag cctgttaaac ggcgttgcaac aatctctctg
 1441 cgcaacgcgt cagtgggcgt atcattaaat atcccgctga tgaccaggat gccattgtctg
 1501 tgggaagctgc ctgcactaat gttccggcgt tatctcttga tgtctctgac cagacaccca
 1561 tcaaacgatat tattttctcc catgaagacg gtaccgcgact gggcgtggag catctgtctgc
 1621 cattgggtca ccagcaaatc gcgctgttag cggggccact aagtctctgt tcggcgcgctc
 1681 tgcgtctggc ttgctggcat aaatatctca ctgcgaatca aattcagccg atagcggaaac
 1741 gggaaggcga ctggagtgcc atgtccgggt ttcaacaacac catgcaaatg ctgaattgagg
 1801 gctgctgtcc cactgcgatg ctgggtggca acgatccgat ggtcttcggc gtaacgcggg
 1861 ccatcacoga gtccggcgct gcgctgggtg cggatattct ggtgatggga tacgacgata
 1921 ccgaagacac atcattttat atcccgccgt taaccacat caaacaggat tttcgctctg
 1981 tggggcaaac cagcgtggac cgcttgcctg aactctctca gggccaggcg gtgaaggcca
 2041 atcagctgtc gccctgtctc ctggtgaaaa gaaaaaccac cctggcgccc aataccgaaa
 2101 ccgcctctccc ccgcggtgtg gcgattcaat taatgcagct tgaccagacg gttctccgac
 2161 tgggaagcgt gcagtgagcg caaccgaatt aatgttaagt agctcactca ttaggcaccc
 2221 cagggctttac actttatgct ccgacgttgc aagaaacctca cgtcagtgcc catctttcgg
 2281 ggaatctgic gcgggaacccc tatctgttta tttttctaaa tacattcaaa tctgtatccg
 2341 ctctcgagac aataaccctg ataaaatgct caataatatt caaaaaggaa gagtattgag
 2401 atcaaacatt tccgtgtcgc ccttattccc tttttctcgg caatttgcct tccctgtttt
 2461 gccaccacag aaacctcggt gaaagttaaa gatgctgaag atcagttggg tgcaacgagc
 2521 ggttacatcc agaactggat ctcaacagcg gtaagatcct tgagagtttt ccgcccgaa
 2581 aacgtttccc aatgatgagc acttttaaa atctgtcata ttgcgcgcta ttactccgta
 2641 ttgacgcgag gcaagagcaa ctccggtcgc gcatacacta ttctcagaat gaacttggtg
 2701 agtacctacc agtcacagaa aagcatctta cggatggcat gacagtgaag gaattatgca
 2761 gtgctgccat aaccatgagt gataaacctg cggccaaact actcttgaca acgatcgga
 2821 gaccgaagga gtaaccacgt tttttgcaca acatggggga tcatgtaact cgccttgatc
 2881 gtggggaaac ggagctgaat gaagccatcc caaacgacga cggctgcctg acgatgcctg
 2941 tagcaatggc aacaacgttg cgcgaacctat taactggcga actacttact ctacgttccc
 3001 ggcaacaatt aatagactgg atggaggcgg ataaagtgc aggaaccaat ctgcctctcg
 3061 cctctccggc ttgctgtgtt attgctgata aatctggagc cggctgagcgt gggctctcgc
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 3181 cggggagtrca ggcaactatg gatgaacgaa atagacgat cgtctgagta ggtgcctcac
 3241 tgattaaaga ttggtaactg tcagaccaag tttactcata tttactttag attgatttaa
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 3361 aaatccctta acgtgagttt tcgtccact gagcgtcaga ccccgtagaa aagatcaaa
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 3481 cgtaccagag ggtggttttg ttgcgggata aagagctacc aactcttttt ccgaaggtaa
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 3841 ccgaaggagg aaaggcgagc aggtatccgg taagcggcag ggtcggaaca ggaagcgca
 3901 cgaggagcgt tccaggggga aacgcctcgt atctttatag tctgtcggg cgtcaggggg
 3961 tctgactcga gcgtcgatct ttgcgtagct cgtcaggggg gcggagccta ttgaaagcc
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 4081 tctctcgtgt atcccctgat tctgtggata accgtattac cgcctttgct tgagctgata
 4141 cgcctcgcgc cagccggaag accgagcgca gcgagtcagt gcagcaggaa cgcggaagag
 4201 gcccaatcac caaacccgct ctcccccgcg gtcggccagt tcatatagc agaatttaatt

FIG. 13C

4261 ctcatgtttt acagctttat atcgactgca cgggtgcacca atgcttcttg cgtcaggcag
4321 ccatcggaag ctgttggtat gctgtgcagg tctgtaaatca ctgcataaatt cgtgtcgctc
4381 aagcgccagc cccgttcttg ataattttt ttgcccgcag atcataacgg ttcttggcaaa
4441 tattctgaaa tgagctgttg acaattaatc atcgctctgt ataattgttg gaattgttgag
4501 cggataacaa ttctacacag gaacacagca tgaattcaga ttccaccatt aaggagctgg
4561 tggcccgagt gctgcagagg ctgtgcgagc gcggcgcgaa gaacgtgctg cctctcggtc
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4681 ctctacctcc caacacgggt accgacgcac tgcgggggag cggggctgtg ggtctgctgc
4741 tgcgcgcgct ggcgacgcac gtgctggttc acctgctggc acgctgcgcg ctctttgtgc
4801 tgggtgtctc cagctgtgcgc taccagggtg cggggcgccc gctgtaccag cgtgtgctgc
4861 ccaactcaggc cgggcccccg ccacacgcct gtggaccocg aaggcgtctg ggtatgcgaac
4921 gggcctggaa ccatagcgtc agggaggccg ggggtccccc gggcctgccca gcccccgggtg
4981 cgaggagcgg cgggggcagt gccagccgaa gtctgcgctg gcccaaggag ccacggcgctg
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5581 cagcagccgg tgctctgtcc cggggaaggc cccagggtct tgtagcgccc cccgaggagg
5641 aggcacacaga ccccccgcgc ctggtgcagc gctctcgcca gccacagcag ccctggcggt
5701 tgtacggctt cgtgcggggt tgcctgcgcc ggtcgtgtcc cccaggccctc tggggctcca
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6781 acttctcag gactcgttcc gaagtgttct gagtatggct gctgtggtga cttagcgagg
6841 acagtgttga acttccctgt agaagacgaa gccttgggtg gcacggcttt tttcagtgag
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7021 ggcttcaagg cgtggaggaa catgcttgc aaactcttgc ggggtctgag gctgaagtg
7081 cacagcgtgt ttctggatt gcagggtgaa agcctccaga cgggtgtcac caactctac
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7201 cagcaagttt ggaagaaccc cacatttttc cctcgcgtca ttctcgacac ggcctccctc
7261 tgcataccca tcccgaagc caagaacgca cccgaagaaa acatttctgt gctgaacctc
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7381 agcttctccg tgctctctg gaggggagt ttgtctggcc ggtgactctc cagcctctgt
7441 ttccccccag gtagtctcgt gggggccaa ggcgcgcg cccctctgct cctcagggac
7501 tgcagctggc tgtccacca agcatctctg ctcaagctga ctgcacacc ttgcacctac
7561 gtgccactcc tggggtcact caggacagc aagtgtgggt ggaggccagt gcggg

D:\Vector NTL\PAK128.4.gb

FIG. 13D

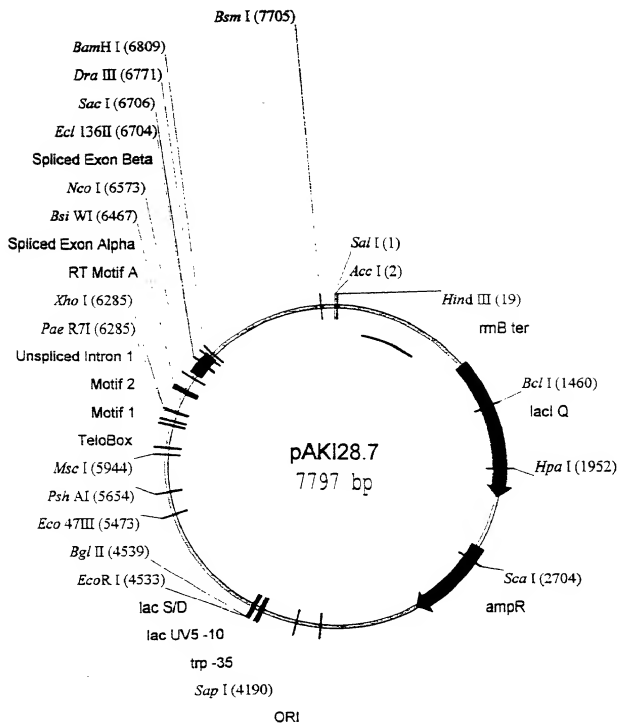


FIG. 14A

LOCUS pAKI28.7 7797 bp dsDNA Circular
 DEFINITION Human telomerase clone with alternative C-terminus

```

1  tgcacctgca ggcattgcaag cttggcactg gccgtcgttt tacaacgtcg tgactgggaa
61  aacctctggc ttacccaact taatcgccct gcagcacatc cccctttcgc cagctggcgt
121 aatagcgaag aggcccgcac cgatcgccct tcccaacagt tgcgcagcct gaatggcgaa
181 tggcgccctga tgcggtattt tctccttacg catctgtgcg gtatttcaca ccgcataaat
241 tccctgtttt ggcggatgag agaagatttt cagcctgata cagattaaat cagaacgcag
301 aagcgtctct ataaaacaga atttgcttgg cggcagtagc gcggtggtcc cacctgaccc
361 catgccgaac tcagaagtga aacgccgtag cgccgatggt agtgtggggt ctcccatgc
421 gagagtaggg aactgccagg catcaataa aacgaaaggc tcagtcgaaa gactgggcct
481 ttctgtttat ctgttgtttg tcggtgaacg ctctcctgag taggacaaat ccgccgggag

```

FIG. 14B

541 cggatttgaa cgttgccaag caacggcccg gagggtggcg ggcaggacgc ccgccataaa
601 ctgcaggcca tcaaatgaag cagaaggcca tcttgacgga tggccttttt cgcctttctac
661 aaactcttcc tgtcgtcata tctacaagcc atcccccac agatagcgga aactagcctc
721 gtttttgcat caggaaagca gggaaatttat ggtgcaactc cagtacaatc tgcctctgatg
781 cgcgcatagt aagccagccc gcacaccccg caacacccgc tgacgcgccc tgacgggctt
841 gctcgtcccc ggcctccgct tacagacaag cctgtgacct cctcgggagc tgcatgtgtc
901 agagggtttt accgtcatca ccgaacacgc cgagacgaaa gggcctcgtg atacgcctat
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1021 ccacggaatg gtgcaaaacc tttcgcggta tggcatgata gcgcgcggaa gagagtcaat
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1141 cttatcagac cgtttccccc gtgtgtgaacc agggccagcca cgtttctgcg aaaaacgcggg
1201 aaaaagtggg agcggcgatg gcgggagctga attacattcc caaccgcgtg gcacaacaac
1261 tggcggggcaa acagtctgtg ctgattggcg ttgccacctc cagttctggcc ctgcacggcg
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1381 tgtcgttggt agaacgaag ggcgtcgaag cctgtaaaag ggcggtgcac aatcttctgc
1441 cgcaacgcgt cagtgggctg atcattaaat atcgcctgga tgacacggat gccattgctg
1501 tgggaagctg tgcgaactat gttccggcgt tatttctgta tgcctctgag ctagcagcca
1561 tcaacagtat tatttctccc catgaagacg gtacgcgact gggcgtggag catctggtct
1621 cattgggtca ccagcaaatc gcgctgttag gcggcccat taaattctgc tgcgcgcgtc
1681 tgcgtctggc tggcgtggat aaatatctca ctgcgaatca aattcagcct atacgcggaac
1741 ggggaaggcca ctggagtgc atgtccgggt tccaacaacac catgcaaatg ctgaatgagg
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2401 attcaacatt tccggtgcgc cettattccc ttttttgcgg caatttgccct tccgtgtttt
2461 gctcacccag aaacgcttgt gaaagtaaaa gatgctgaag atcagttggg tgcaacgagt
2521 ggtttacatcg agaactggat cccaacagcg gtaagtacct tgagagtttt cgcctccgag
2581 aacgttttcc aatgatgagc acttttaaa tctcgtatg tggcgggta tctcccgta
2641 ttgacgcggc gcaagagcaa cctcggctgc gcatacacta ttctcagatg gaacttggtt
2701 agtactcacc agtcacagaa agcatctcta cggatggcat gacagtgaag gaattatgca
2761 gtgctgccc atccatgagt gataacactg gcggccaaact acttctgaac acgatcgag
2821 gaccgaagga gctaaacgct tttttgcaca acatggggga ctatgtaact cgccttgatc
2881 gttgggaacc ggaagctgaat gaagccatac caaacgcgca gcgtgacacc acgatgctg
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3601 accacttcaa gaactctgta gcaccgccta catactctgc tctgctaact ctgttaccag
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3901 cgaggagact tccaggggga aacgcctggt atctttatat tctgtcggg ttcgccaccc

FIG. 14C

3961 tctgacttga gctgctattt ttgtgatgct cgtcaggggg ggggagccca tggaaaaacg
4021 ccagcaacgc ggccttttta cgttctctgg cctttttctg ccttttttgc caocatgtct
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6961 aaacttcttc aggaactggg ccgaagtgtc ctgagttagg ctgcgtgggt aacttgcgga
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7261 gtcacagcct gtttctggat ttgcaggtga acagcctcca gacgggtgtc accaaccat
7321 acaagactct cctgtgcgag cgttacaggc ttcacgcagc tgtctcgcag cctccatttc

FIG. 14D

7381 atcagcaagt ttggaagaac cccacatttt tcctgcgcgt catctctgac acggcctccc
 7441 tctgctactc catcctgaaa gccagaacg cagccgaaga aaacatttct gtcgtgactc
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 7681 ccgtgcagtg gctgtgccac caagcattcc tgctcaagct gactcgacac cgtgtcacct
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FIG. 14E

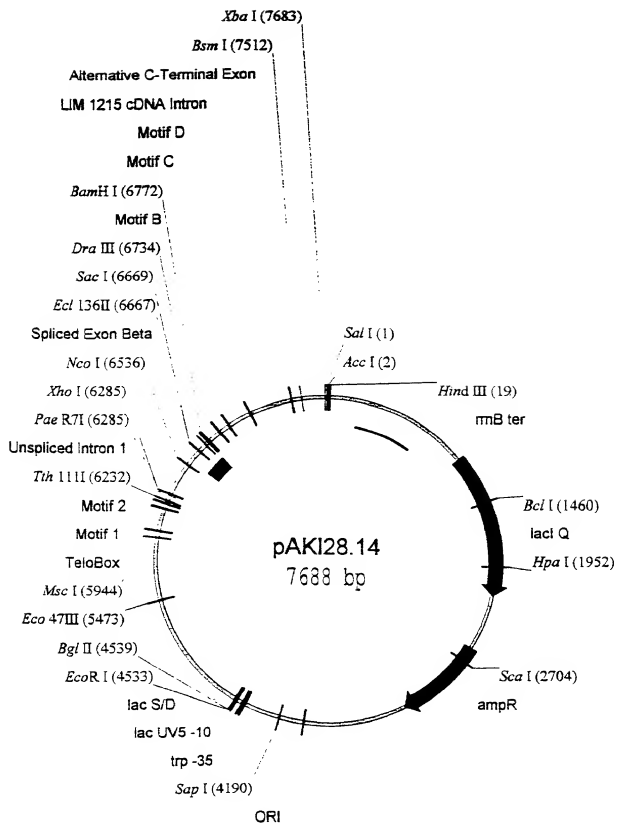


FIG. 15A

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121  aatagcggaag  agggccgcac  cgatgcgcct  tcaccaacagt  tggcgcagcct  gaattggcgaa
181  tggcgccctga  tggcggtatct  tctcctttag  catctgtgcg  gtatttcaca  cgcataaaat
241  tccctgtttt  ggcggatgag  agaagatctt  cagcctgata  cagattaaat  cagaacgcag
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421  gagagtgggt  aactgccagg  catcaataaa  aacgaaaggc  tcagtgcgaa  gactggcgct
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661  aaactcttcc  tgtcgtcata  tctacaagcc  atccccccac  agatacggta  aactagcctc
721  ctgttttgcat  caggaaagca  gggaatttat  ggtgcactct  cagtacaatc  tgctctgatg
781  cgcgatagtt  aagccagccc  cgacaccocg  caacaccocg  tgacgcgccc  tgacgggctt
841  gtctgctccc  ggcattccgt  tacagacaag  ctgtgacctg  ctcccgggag  tgcatgtgtc
901  agaggttttc  accgtcatca  ccgaaacgcg  cgagacgaaa  gggcctcgtg  ataccgctat
961  ttttataagt  taatgtcatg  ataataatgg  tttcttagac  gtgaggttct  gtacccgcac
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1261  tggcgggcaaa  acagtctgtt  ctgatttggc  ctgctacctc  cagttcgccc  ctgcacgcgc
1321  cgtgcgaaat  tgtcggcgcg  attaaaatcc  gcgcgagcca  actgggtggc  agcgtgtgtg
1381  tgtcgtagtt  agaacgaaag  ggcgttgaag  cctgtaaaag  ggcggtgcac  aaatcttctg
1441  cgcacagctgt  cagctggcgt  atcaataact  atcctctgga  tgaccaggat  gccattctgt
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3001  ggcaacaatt  aatagactgg  atggagcgcg  aaaaagtgc  aggaacactt  ctgcgtctcg
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3241  tgattaaagca  ttggttaact  tcagaccaga  ttaactcata  tataacttag  attgatttaa
3301  aactctaatc  ttaatttaaa  aggatctagg  ttaagtata  ttttgataat  ctcatgacca

```

FIG. 15B

3361 aaatccctta acgtgagttt tegtccact gagcgtcaga ccccgtagaa aagatcaaa
3421 gatctctctt agatcccttt ttctctcgccg taatctgctg cttgcacaaca aaaaaaccac
3481 cgtccaccagc ggtggtttgt ttgcgggagc aagagctacc aactcttttt cogaaggtaa
3541 ctggtcttcag cagagcgcag ataccaaata ctgtccttct agtgtagccg tagttaggcc
3601 accacttcaa gaactctgta gcaaccgcta ctactctgc ttgctaactc cgtttaccag
3661 tggctgctgc cagtggcgat aagtctgtgt ttaccgggtt ggactcaaga cगतtagttac
3721 cggataaagg cgacgggtcg ggtctgaacgg ggggttcgtg cacacagccc agcttggagg
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4861 cccctcagcy ccggcccccg accacgccta gtggaccocg aagcgctctg ggtatgcgaac
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6601 gcagacgttc gttgctcaacc tgcaaggagac cagcccgctg agggatgcgc tegtatcga
6661 gcagagctcc tccctggaat agggcagcag ttgctctctc cagctctctc tacgctctca
6721 gtgccaccac gcgctgcgca tcaaggggaa tgcctcagtc cagtcccgag ggaatccgca

FIG. 15C

6781 gggctccatc ctctccacgc tgcctctgcag cctgtgctac ggcgacatgg agaacaagct
 6841 gttttgcggg attcggcggg acgggctgct cctgcgtttg gtggatgatt tcttgttggg
 6901 gacacctcac ctccaccacg cgaaaacctt cctcaggacc ctggtccgag gtgtccctga
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 7381 gcgcgtcac tctgacacgg cctccctctg ctactccatc ctgaaagcca agaacgcagg
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 7621 tgcctggag gccgcagcca acccggcact gccctcagac ttcaagacca tctcggaactg
 7681 atctagag

FIG. 15D

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Present Application:

Applicants : Andrzej Kilian and David Bowtell
Title : VERTEBRATE TELOMERASE GENES AND PROTEINS AND
USES THEREOF
Docket No. : 190106.407C1
Date : February 11, 2000

Prior Application:

Examiner : Einar Stole, Ph.D.
Art Unit : 1653
Application No.: 09/108,401

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION

Sir:

I, Lawrence Teague, in accordance with 37 C.F.R. § 1.821(f) do hereby declare that, to the best of my knowledge, the content of the paper entitled "Sequence Listing" enclosed herewith and the computer readable copy contained within the floppy disk filed in U.S. Patent Application Number 09/108,401 filed June 30, 1998, are the same.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated this 11th day of February, 2000.


Lawrence Teague
Biotechnology Paralegal

701 5th Ave, Suite 6300
Seattle, WA 98104-7092
(206) 622-4900
FAX (206) 682-6031

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Andrzej Kilian and David Bowtell
Title : VERTEBRATE TELOMERASE GENES AND PROTEINS AND
USES THEREOF
Filed : February 11, 2000

Docket No. : 190106.407C1
Date : February 11, 2000

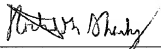
Box Patent Application
Assistant Commissioner for Patents
Washington, DC 20231

APPOINTMENT OF ASSOCIATE POWER OF ATTORNEY

Assistant Commissioner for Patents:

I, Richard G. Sharkey, Ph.D., attorney of record in the above-identified application, appoint as associate attorney William T. Christiansen, Ph.D., Registration No. 44,614, of the firm of Seed Intellectual Property Law Group PLLC, 701 Fifth Avenue, Suite 6300, Seattle, Washington 98104-7092.

Respectfully submitted,
Seed Intellectual Property Law Group PLLC



Richard G. Sharkey, Ph.D.
Registration No.

WTC:rap

701 Fifth Avenue, Suite 6300
Seattle, Washington 98104-7092
Phone: (206) 622-4900
Fax: (206) 682-6031

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DECLARATION

As the below-named inventors, we declare that:

Our residences, post office addresses, and citizenships are as stated below under our names.

We believe we are the original, first, and joint inventors of the invention entitled "VERTEBRATE TELOMERASE GENES AND PROTEINS AND USES THEREOF," which is described and claimed in the specification and claims of Patent Application No. 09/108,401, which we filed in the United States Patent and Trademark Office on June 30, 1998 and for which a patent is sought. This application claims the benefit of Provisional Application Nos. 60/051,410, filed July 1, 1997; 60/053,018, filed July 19, 1997; 60/053,329, filed July 21, 1997; 60/054,642, filed August 4, 1997; and 60/058,287, filed September 9, 1997.

We have reviewed and understand the contents of the above-entitled specification, including the claims, as amended by any amendment specifically referred to herein (if any).

We acknowledge our duty to disclose information of which we are aware which is material to the patentability and examination of this application in accordance with 37 C.F.R. § 1.56(a).

We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that the making of willfully false statements and the like is punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and may jeopardize the validity of any patent issuing from this patent application.


Andrzej Killan

Date

2/10/98

Residence

: City of Canberra, ACT
Country of Australia

Citizenship

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David Bowtell

Date

21st October 1998Residence : City of Coburg, Victoria
Country of Australia

Citizenship : Australia

P.O. Address : 22 Strathcarr Avenue
Coburg, Victoria
AUSTRALIA

(190106.407)

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Andrzej Kilian and David Bowtell
 Application No. : 09/108,401
 Filed : June 30, 1998
 For : VERTEBRATE TELOMERASE GENES AND PROTEINS AND
 USES THEREOF

Docket No. : 190106.407

Box Missing Parts
 Assistant Commissioner for Patents
 Washington, DC 20231

ELECTION UNDER 37 C.F.R. §§ 3.71 AND 3.73 AND POWER OF ATTORNEY

The undersigned, being Co-Assignee of the interest in the above-identified application by virtue of an Assignment which is being filed concurrently herewith or has already been filed, hereby elects, under 37 C.F.R. § 3.71, to prosecute the application to the exclusion of the inventors.

Assignee hereby appoints RICHARD W. SEED, Reg. No. 16,557; ROBERT J. BAYNHAM, Reg. No. 22,846; EDWARD W. BULCHIS, Reg. No. 26,847; GEORGE C. RONDEAU, JR., Reg. No. 28,893; DAVID H. DEITS, Reg. No. 28,066; WILLIAM O. FERRON, JR., Reg. No. 30,633; PAUL T. MEIKLEJOHN, Reg. No. 26,569; DAVID J. MAKI, Reg. No. 31,392; RICHARD G. SHARKEY, Reg. No. 32,629; DAVID V. CARLSON, Reg. No. 31,153; MAURICE J. PIRIO, Reg. No. 33,273; KARL R. HERMANN, Reg. No. 33,507; DAVID D. MCMASTERS, Reg. No. 33,963; ROBERT IANNUCCI, Reg. No. 33,514; MICHAEL J. DONOHUE, Reg. No. 35,859; CHRISTOPHER J. DALEY-WATSON, Reg. No. 34,807; STEVEN D. LAWRENZ, Reg. No. 37,376; ROBERT G. WOOLSTON, Reg. No. 37,263; ELLEN M. BIERMAN, Reg. No. 38,079; BRYAN A. SANTARELLI, Reg. No. 37,560; CAROL NOTTENBURG, Reg. No. 39,317; CRAIG S. JEPSON, Reg. No. 33,517; PAUL T. PARKER, Reg. No. 38,264; JOHN C. STEWART, Reg. No. 40,188; DAVID W. PARKER, Reg. No. 37,414; ROBERT E. MATES, Reg. No. 35,271; BRIAN G. BODINE, Reg. No. 40,520; FRANK ABRAMONTE, Reg. No. 38,066; E. RUSSELL TARLETON, Reg. No. 31,800; FREDERICK M. FLIEGEL, Reg. No. 36,138; JAN C. L. MAXWELL, Reg. No. 41,181;

THOMAS L. EWING, Reg. No. 34,328; CLIFTON G. GREEN, Reg. No. 41,044; KEVIN S. COSTANZA, Reg. No. 37,301; DALE C. BARR, Reg. No. 40,498; KEVIN S. ROSS, Reg. No. P-42,116; PAUL F. RUSYN, Reg. No. P-42,118; JOHN M. WECHKIN, Reg. No. P-42,216; THOMAS E. LOOP, Reg. No. P-42,810; STEPHEN J. ROSENMAN, Reg. No. P-43,058; and BRIAN L. JOHNSON, Reg. No. 40,033, comprising the firm of SEED AND BERRY LLP, 6300 Columbia Center, Seattle, Washington 98104-7092, as its attorneys to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. Please direct all telephone calls to David D. McMasters at (206) 622-4900 and telecopies to (206) 682-6031.

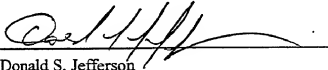
Please direct all communications to:

David D. McMasters, Esq.
Seed and Berry LLP
6300 Columbia Tower
701 Fifth Avenue
Seattle, Washington 98104-7092

Pursuant to 37 C.F.R. § 3.73, the undersigned duly authorized designee of Assignee certifies that the evidentiary documents have been reviewed, specifically the Assignment to Cambia Biosystems LLC filed concurrently herewith for recording, a copy of which is attached hereto, and certifies that to the best of my knowledge and belief, title remains in the name of the Assignee.

Cambia Biosystems LLC
ASSIGNEE

10/12/98
DATE


Donald S. Jefferson
Managing Member

Enclosure:
Copy of Assignment

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Andrzej Kilian and David Bowtell
 Application No. : 09/108,401
 Filed : June 30, 1998
 For : VERTEBRATE TELOMERASE GENES AND PROTEINS AND
 USES THEREOF

Docket No. : 190106.407

Box Missing Parts
 Assistant Commissioner for Patents
 Washington, DC 20231

ELECTION UNDER 37 C.F.R. §§ 3.71 AND 3.73 AND POWER OF ATTORNEY

The undersigned, being Co-Assignee of the interest in the above-identified application by virtue of an Assignment which is being filed concurrently herewith or has already been filed, hereby elects, under 37 C.F.R. § 3.71, to prosecute the application to the exclusion of the inventors.

Assignee hereby appoints RICHARD W. SEED, Reg. No. 16,557; ROBERT J. BAYNHAM, Reg. No. 22,846; EDWARD W. BULCHIS, Reg. No. 26,847; GEORGE C. RONDEAU, JR., Reg. No. 28,893; DAVID H. DEITS, Reg. No. 28,066; WILLIAM O. FERRON, JR., Reg. No. 30,633; PAUL T. MEIKLEJOHN, Reg. No. 26,569; DAVID J. MAKI, Reg. No. 31,392; RICHARD G. SHARKEY, Reg. No. 32,629; DAVID V. CARLSON, Reg. No. 31,153; MAURICE J. PIRIO, Reg. No. 33,273; KARL R. HERMANN, Reg. No. 33,507; DAVID D. MCMASTERS, Reg. No. 33,963; MICHAEL J. DONOHUE, Reg. No. 35,859; CHRISTOPHER J. DALEY-WATSON, Reg. No. 34,807; STEVEN D. LAWRENZ, Reg. No. 37,376; ROBERT G. WOOLSTON, Reg. No. 37,263; ELLEN M. BIERMAN, Reg. No. 38,079; PAUL T. PARKER, Reg. No. 38,264; JOHN C. STEWART, Reg. No. 40,188; DAVID W. PARKER, Reg. No. 37,414; BRIAN G. BODINE, Reg. No. 40,520; FRANK ABRAMONTE, Reg. No. 38,066; E. RUSSELL TARLETON, Reg. No. 31,800; FREDERICK M. FLIEGEL, Reg. No. 36,138; JAN CAROL LITTLE, Reg. No. 41,181; THOMAS L. EWING, Reg. No. 34,328; CLIFTON G. GREEN, Reg. No. 41,044; KEVIN S. COSTANZA, Reg. No. 37,801; DALE C. BARR, Reg. No. 40,498; KEVIN S. ROSS, Reg. No. 42,116; PAUL F. RUSYN, Reg. No. 42,118; JOHN M.

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Please direct all communications to:

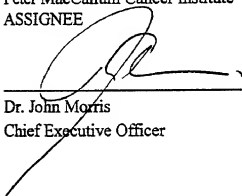
David D. McMasters, Esq.
Seed and Berry LLP
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Pursuant to 37 C.F.R. § 3.73, the undersigned duly authorized designee of Assignee certifies that the evidentiary documents have been reviewed, specifically the Assignment to Cambia Biosystems LLC filed concurrently herewith for recording, a copy of which is attached hereto, and certifies that to the best of my knowledge and belief, title remains in the name of the Assignee.

Peter MacCallum Cancer Institute
ASSIGNEE

13/10/98

DATE



Dr. John Morris
Chief Executive Officer

Enclosure:
Copy of Assignment

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